8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, AND 67084 ALT, HUMAN PROTEINS AND METHODS OF USE THEREOF

Related Applications

This application claims the benefit of prior-filed provisional patent application Serial No. 60/256,240, filed December 15, 2000, entitled "8099 AND 46455, NOVEL HUMAN SUGAR TRANSPORTERS AND USES THEREFOR," prior-filed provisional patent application Serial No. 60/256,588, filed December 18, 2000, entitled "54414 AND 53763, NOVEL HUMAN POTASSIUM CHANNELS AND USES THEREFOR," and prior-filed provisional patent application Serial No. 60/258,028, filed December 21, 2000, entitled "67076, 67102, 44181, 67084FL, and 67084alt, NOVEL HUMAN PHOSPHOLIPID TRANSPORTERS AND USES THEREOF." The entire contents of the above-referenced applications are incorporated herein by this reference.

Background of the Invention

Cellular membranes serve to differentiate the contents of a cell from the surrounding environment, and may also serve as effective barriers against the unregulated influx of hazardous or unwanted compounds, and the unregulated efflux of desirable compounds. Membranes are by nature impervious to the unfacilitated diffusion of hydrophilic compounds such as proteins, water molecules, and ions due to their structure: a bilayer of lipid molecules in which the polar head groups face outward (towards the exterior and interior of the cell) and the nonpolar tails face inward (at the center of bilayer, forming a hydrophobic core). Membranes enable a cell to maintain a relatively higher intracellular concentration of desired compounds and a relatively lower intracellular concentration of undesired compounds than are contained within the surrounding environment.

Membranes also present a structural difficulty for cells, in that most desired compounds cannot readily enter the cell, nor can most waste products readily exit the cell through this lipid bilayer. The import and export of such compounds is regulated by proteins which are embedded (singly or in complexes) in the cellular membrane. Two mechanisms exists whereby membrane proteins allow the passage of compounds: non-mediated and mediated transport. Simple diffusion is an example of non-mediated transport, while facilitated diffusion and active transport are examples of mediated transport. Permeases, porters, translocases, translocators, and transporters are proteins that engage in mediated transport (Voet and Voet (1990) Biochemistry, John Wiley and Sons, Inc., New York, N.Y. pp. 484-505).

Sugar transporters are members of the major facilitator superfamily of transporters. These transporters are passive in the sense that they are driven by the substrate concentration

gradient and they exhibit distinct kinetics as well as sugar substrate specificity. Members of this family share several characteristics: (1) they contain twelve transmembrane domains separated by hydrophilic loops; (2) they have intracellular N- and C-termini; and (3) they are thought to function as oscillating pores. The transport mechanism occurs via sugar binding to the exofacial binding site of the transporter, which is thought to trigger a conformational change causing the sugar binding site to re-orient to the endofacial conformation, allowing the release of substrate. These transporters are specific for various sugars and are found in both prokaryotes and eukaryotes. In mammals, sugar transporters transport various monosaccharides across the cell membrane (Walmsley *et al.* (1998) *Trends in Biochem. Sci.* 23:476-481; Barrett *et al.* (1999) *Curr. Op. Cell Biol.* 11:496-502).

At least nine mammalian glucose transporters have been identified, GLUT1 - GLUT9, which are expressed in a tissue-specific manner (*e.g.*, in brain, erythrocyte, kidney, muscle, and adipose tissues) (Shepherd *et al.* (1999) *N. Engl. J. Med.* 341:248-257; Doege *et al.* (2000) *Biochem. J.* 350:771-776). Some GLUT proteins have been shown to be present in low amounts at the plasma membrane during the basal state, at which time large amounts are sequestered in intracellular vesicle stores. Stimulatory molecules specific for each GLUT (such as insulin) regulate the translocation of the GLUT-containing vesicles to the plasma membrane. The vesicles fuse at the membrane and subsequently expose the GLUT protein to the extracellular milieu to allow glucose (and other monosaccharide) transport into the cell (Walmsley *et al.* (1998) *Trends in Biochem. Sci.* 23:476-481; Barrett *et al.* (1999) *Curr. Op. Cell Biol.* 11:496-502). Other GLUT transporters play a role in constitutive sugar transport.

Potassium (K^+) channels are ubiquitous proteins which are involved in the setting of the resting membrane potential as well as in the modulation of the electrical activity of cells. In excitable cells, K^+ channels influence action potential waveforms, firing frequency, and neurotransmitter secretion (Rudy, B. (1988) *Neuroscience*, 25, 729-749; Hille, B. (1992) *Ionic Channels of Excitable Membranes*, 2nd Ed.). In non-excitable cells, they are involved in hormone secretion, cell volume regulation and potentially in cell proliferation and differentiation (Lewis *et al.* (1995) *Annu. Rev. Immunol.*, 13, 623-653). Developments in electrophysiology have allowed the identification and the characterization of an astonishing variety of K^+ channels that differ in their biophysical properties, pharmacology, regulation and tissue distribution (Rudy, B. (1988) *Neuroscience*, 25, 729-749; Hille, B. (1992) *Ionic Channels of Excitable Membranes*, 2nd Ed.). More recently, cloning efforts have shed considerable light on the mechanisms that determine this functional diversity. Furthermore, analyses of structure-function relationships have provided an important set of data concerning the molecular basis of the biophysical properties (selectivity, gating, assembly) and the pharmacological properties of cloned K^+ channels.

Functional diversity of K^+ channels arises mainly from the existence of a great number of genes coding for pore-forming subunits, as well as for other associated regulatory subunits. Two main structural families of pore-forming subunits have been identified. The first one consists of subunits with a conserved hydrophobic core containing six transmembrane domains (TMDs). These K^+ channel α subunits participate in the formation of outward rectifier voltage-gated (Kv) and Ca^{2+}-dependent K^+ channels. The fourth TMD contains repeated positive charges involved in the voltage gating of these channels and hence in their outward rectification (Logothetis *et al.* (1992) *Neuron,* 8, 531-540; Bezanilla *et al.* (1994) *Biophys. J.* 66, 1011-1021).

The second family of pore-forming subunits have only two TMDs. They are essential subunits of inward-rectifying (IRK), G-protein-coupled (GIRK) and ATP-sensitive (K_{ATP}) K^+ channels. The inward rectification results from a voltage-dependent block by cytoplasmic Mg^{2+} and polyamines (Matsuda, H. (1991) *Annu. Rev. Physiol.*, 53, 289-298). A conserved domain, called the P domain, is present in all members of both families (Pongs, O. (1993) *J. Membr. Biol.*, 136, 1-8; Heginbotham *et al.* (1994) *Biophys. J.* 66,1061-1067; Mackinnon, R. (1995) *Neuron*, 14, 889-892; Pascual *et al.*, (1995) *Neuron.*, and 14, 1055-1063). This domain is an essential element of the aqueous K^+-selective pore. In both groups, the assembly of four subunits is necessary to form a functional K^+ channel (Mackinnon, R. (1991) *Nature*, 350, 232-235; Yang *et al.*, (1995) *Neuron*, 15, 1441-1447.

In both six TMD and two TMD pore-forming subunit families, different subunits coded by different genes can associate to form heterotetramers with new channel properties (Isacoff *et al.*, (1990) *Nature*, 345, 530-534). A selective formation of heteropolymeric channels may allow each cell to develop the best K^+ current repertoire suited to its function. Pore-forming α subunits of Kv channels are classified into different subfamilies according to their sequence similarity (Chandy *et al.* (1993) *Trends Pharmacol. Sci.*, 14: 434). Tetramerization is believed to occur preferentially between members of each subgroup (Covarrubias *et al.* (1991) *Neuron*, 7, 763-773). The domain responsible for this selective association is localized in the N-terminal region and is conserved between members of the same subgroup. This domain is necessary for hetero- but not homo-multimeric assembly within a subfamily and prevents co-assembly between subfamilies. Recently, pore-forming subunits with two TMDs were also shown to co-assemble to form heteropolymers (Duprat *et al.* (1995) *Biochem. Biophys. Res. Commun.*, 212, 657-663. This heteropolymerization seems necessary to give functional GIRKs. IRKs are active as homopolymers but also form heteropolymers.

New structural types of K^+ channels were identified recently in both humans and yeast. These channels have two P domains in their functional subunit instead of only one (Ketchum *et al.* (1995) *Nature*, 376, 690-695; Lesage *et al.* (1996) *J. Biol. Chem.*, 271, 4183-4187; Lesage *et al.* (1996) *EMBO J.*, 15, 1004-1011; Reid *et al.* (1996) *Receptors*

Channels 4, 51-62). The human channel called TWIK-1, has four TMDs. TWIK-1 is expressed widely in human tissues and is particularly abundant in the heart and the brain. TWIK-1 currents are time independent and inwardly rectifying. These properties suggest that TWIK-1 channels are involved in the control of the background K^+ membrane conductance (Lesage *et al.* (1996) *EMBO J.*, 15, 1004-1011).

Potassium channels are potassium ion selective, and can determine membrane excitability (the ability of, for example, a neuron to respond to a stimulus and convert it into an impulse). Potassium channels can also influence the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation. Potassium channels are typically expressed in electrically excitable cells, *e.g.*, neurons, muscle, endocrine, and egg cells, and may form heteromultimeric structures, *e.g.*, composed of pore-forming and cytoplasmic subunits. Potassium channels may also be found in non-excitable cells, where they may play a role in, *e.g.*, signal transduction. Examples of potassium channels include: (1) the voltage-gated potassium channels, (2) the ligand-gated potassium channels, *e.g.*, neurotransmitter-gated potassium channels, and (3) cyclic-nucleotide-gated potassium channels. Voltage-gated and ligand-gated potassium channels are expressed in the brain, *e.g.*, in brainstem monoaminergic and forebrain cholinergic neurons, where they are involved in the release of neurotransmitters, or in the dendrites of hippocampal and neocortical pyramidal cells, where they are involved in the processes of learning and memory formation. For a detailed description of potassium channels, see Kandel E. R. *et al.*, Principles of Neural Science, second edition, (Elsevier Science Publishing Co., Inc., N.Y. (1985)), the contents of which are incorporated herein by reference.

The E1-E2 ATPase family is a large superfamily of transport enzymes that contains at least 80 members found in diverse organisms such as bacteria, archaea, and eukaryotes (Palmgren, M. G. and Axelsen, K. B. (1998) *Biochim. Biophys. Acta.* 1365:37-45). These enzymes are involved in ATP hydrolysis-dependent transmembrane movement of a variety of inorganic cations (*e.g.*, H^+, Na^+, K^+, Ca^{2+}, Cu^{2+}, Cd^+, and Mg^{2+} ions) across a concentration gradient, whereby the enzyme converts the free energy of ATP hydrolysis into electrochemical ion gradients. E1-E2 ATPases are also known as "P-type" ATPases, referring to the existence of a covalent high-energy phosphoryl-enzyme intermediate in the chemical reaction pathway of these transporters. Until recently, the superfamily contained four major groups: Ca^{2+} transporting ATPases; Na^+/K^+- and gastric H^+/K^+ transporting ATPases; plasma membrane H^+ transporting ATPases of plants, fungi, and lower eukaryotes; and all bacterial P-type ATPases (Kuhlbrandt *et al.* (1998) *Curr. Opin. Struct. Biol.* 8:510-516).

E1-E2 ATPases are phosphorylated at a highly conserved DKTG sequence. Phosphorylation at this site is thought to control the enzyme's substrate affinity. Most E1-E2 ATPases contain ten alpha-helical transmembrane domains, although additional domains

may be present. A majority of known gated-pore translocators contain twelve alpha-helices, including Na^+/H^+ antiporters (West (1997) *Biochim. Biophys. Acta* 1331:213-234).

Members of the E1-E2 ATPase superfamily are able to generate electrochemical ion gradients which enable a variety of processes in the cell such as absorption, secretion, transmembrane signaling, nerve impulse transmission, excitation/contraction coupling, and growth and differentiation (Scarborough (1999) *Curr. Op. Cell Biol.* 11:517-522). These molecules are thus critical to normal cell function and well-being of the organism.

Recently, a new class of E1-E2 ATPases was identified, the aminophospholipid transporters or translocators. These transporters transport not cations, but phospholipids (Tang, X. et al. (1996) *Science* 272:1495-1497; Bull, L. N. et al. (1998) *Nat. Genet.* 18:219-224; Mauro, I. et al. (1999) *Biochem. Biophys. Res. Commun.* 257:333-339). These transporters are involved in cellular functions including bile acid secretion and maintenance of the asymmetrical integrity of the plasma membrane.

Given the important biological and physiological roles played by the sugar transporter family of proteins, the potassium channel family of proteins, and the E1-E2 ATPase family of proteins, there exists a need to identify novel potassium channel family members for use in a variety of diagnostic/prognostic, as well as therapeutic applications

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel human sugar transporter family members, referred to herein as "8099 and 46455" nucleic acid and polypeptide molecules. The 8099 and 46455 nucleic acid and polypeptide molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes, *e.g.*, sugar homeostasis. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding 8099 and 46455 polypeptides or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of 8099 and 46455-encoding nucleic acids.

The present invention is also based, at least in part, on the discovery of novel potassium channel family members, referred to herein as "54414 and 53763" nucleic acid and polypeptide molecules. The 54414 and 53763 nucleic acid and protein molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes, *e.g.*, gene expression, intra- or intercellular signaling, and/or membrane excitability or conductance. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding 54414 and 53763 proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of 54414 and 53763-encoding nucleic acids.

The present invention is also based, at least in part, on the discovery of novel human phospholipid transporter family members, referred to herein as "67076, 67102, 44181,

67084FL, or 67084alt" nucleic acid and polypeptide molecules. The 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid and polypeptide molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes, *e.g.*, phospholipid transport (*e.g.*, aminophospholipid transport), absorption, secretion, gene expression, intra- or inter-cellular signaling, and/or cellular proliferation, growth, apoptosis, and/or differentiation. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding 67076, 67102, 44181, 67084FL, or 67084alt polypeptides or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of 67076, 67102, 44181, 67084FL, or 67084alt-encoding nucleic acids.

In one embodiment, the invention features an isolated nucleic acid molecule that includes the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:27. In another embodiment, the invention features an isolated nucleic acid molecule that encodes a polypeptide including the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26. In another embodiment, the invention features an isolated nucleic acid molecule that includes the nucleotide sequence contained in the plasmid deposited with ATCC® as Accession Number _____, _____, _____, _____, or _____.

In still other embodiments, the invention features isolated nucleic acid molecules including nucleotide sequences that are substantially identical (*e.g.*, 60% identical) to the nucleotide sequence set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:27. The invention further features isolated nucleic acid molecules including at least 50 contiguous nucleotides of the nucleotide sequence set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:27. In another embodiment, the invention features isolated nucleic acid molecules which encode a polypeptide including an amino acid sequence that is substantially identical (*e.g.*, 60% identical) to the amino acid sequence set forth as SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26. The present invention also features nucleic acid molecules which encode allelic variants of the

polypeptide having the amino acid sequence set forth as SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26. In addition to isolated nucleic acid molecules encoding full-length polypeptides, the present invention also features nucleic acid molecules which encode fragments, for example, biologically active or antigenic fragments, of the full-length polypeptides of the present invention (*e.g.*, fragments including at least 10 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26). In still other embodiments, the invention features nucleic acid molecules that are complementary to, antisense to, or hybridize under stringent conditions to the isolated nucleic acid molecules described herein.

In another aspect, the invention provides vectors including the isolated nucleic acid molecules described herein (*e.g.*, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt-encoding nucleic acid molecules). Such vectors can optionally include nucleotide sequences encoding heterologous polypeptides. Also featured are host cells including such vectors (*e.g.*, host cells including vectors suitable for producing 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules and polypeptides).

In another aspect, the invention features isolated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides and/or biologically active or antigenic fragments thereof. Exemplary embodiments feature a polypeptide including the amino acid sequence set forth as SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, a polypeptide including an amino acid sequence at least 60% identical to the amino acid sequence set forth as SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, a polypeptide encoded by a nucleic acid molecule including a nucleotide sequence at least 60% identical to the nucleotide sequence set forth as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:27. Also featured are fragments of the full-length polypeptides described herein (*e.g.*, fragments including at least 10 contiguous amino acid residues of the sequence set forth as SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26) as well as allelic variants of the polypeptide having the amino acid sequence set forth as SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26.

The 8099 and 46455 polypeptides and/or biologically active or antigenic fragments thereof, are useful, for example, as reagents or targets in assays applicable to treatment and/or diagnosis of 8099 and 46455 mediated or related disorders. In one embodiment, 8099 and/or 46455 polypeptides or fragments thereof, have an 8099 and/or 46455 activity. In another embodiment, 8099 and/or 46455 polypeptides or fragments thereof, have at least one, preferably two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve transmembrane domains and/or a sugar transporter family domain, and optionally, have an 8099 and/or 46455 activity.

The 54414 and 53763 polypeptides and/or biologically active or antigenic fragments thereof, are useful, for example, as reagents or targets in assays applicable to treatment and/or diagnosis of 54414 and 53763 mediated or related disorders. In one embodiment, a 54414 AND 53763 polypeptide or fragment thereof has a 54414 and 53763 activity. In another embodiment, a 54414 and 53763 polypeptide or fragment thereof has at least one or more of the following domains or motifs: a transmembrane domain, an ion transport protein domain, a K^+ channel tetramerisation domain, a P-loop motif, a pore domain, a potassium channel signature sequence motif, and/or a voltage sensor motif, and optionally, has a 54414 or 53763 activity.

The 67076, 67102, 44181, 67084FL, or 67084alt polypeptides and/or biologically active or antigenic fragments thereof, are useful, for example, as reagents or targets in assays applicable to treatment and/or diagnosis of 67076, 67102, 44181, 67084FL, or 67084alt associated or related disorders. In one embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or fragment thereof, has a 67076, 67102, 44181, 67084FL, or 67084alt activity. In another embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or fragment thereof, includes at least one of the following domains, sites, or motifs: a transmembrane domain, an N-terminal large extramembrane domain, a C-terminal large extramembrane domain, an E1-E2 ATPases phosphorylation site, a P-type ATPase sequence 1 motif, a P-type ATPase sequence 2 motif, a P-type ATPase sequence 3 motif, and/or one or more phospholipid transporter specific amino acid resides, and optionally, has a 67076, 67102, 44181, 67084FL, or 67084alt activity.

In a related aspect, the invention features antibodies (*e.g.*, antibodies which specifically bind to any one of the polypeptides described herein) as well as fusion polypeptides including all or a fragment of a polypeptide described herein.

The present invention further features methods for detecting 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides and/or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules, such methods featuring, for example, a probe, primer or antibody described herein. Also featured are kits, *e.g.*, kits for the detection of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides and/or 8099, 46455, 54414, 53763, 67076, 67102,

44181, 67084FL, or 67084alt nucleic acid molecules. In a related aspect, the invention features methods for identifying compounds which bind to and/or modulate the activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule described herein. Further featured are methods for modulating a 67076, 67102, 44181, 67084FL, or 67084alt activity.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figures 1A-1B depict the cDNA sequence and predicted amino acid sequence of human 8099. The nucleotide sequence corresponds to nucleic acids 1 to 2725 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 617 of SEQ ID NO:2. The coding region without the 5' and 3' untranslated regions of the human 8099 gene is shown in SEQ ID NO: 3.

Figure 2 depicts a structural, hydrophobicity, and antigenicity analysis of the human 8099 polypeptide (SEQ ID NO:2).

Figure 3A-C depicts the results of a search which was performed against the HMM database in PFAM.

Figure 4 depicts an alignment of the human 8099 amino acid sequence (SEQ ID NO:2) with the amino acid sequence of the *E. coli* galactose-proton symporter GALP using the CLUSTAL W (1.74) alignment program (having GenBank Accession No. P37021, set forth as SEQ ID NO:28).

Figure 5 depicts an alignment of the human 8099 amino acid sequence (SEQ ID NO:2) with the amino acid sequence of the *E. coli* arabinose-proton symporter ARAE using the CLUSTAL W (1.74) alignment program (having GenBank Accession No. P09830, set forth as SEQ ID NO:29).

*Figure 6 d*epicts an alignment of the human 8099 amino acid sequence (SEQ ID NO:2) with the amino acid sequence of *E. coli* GALP and ARAE using the CLUSTAL W (1.74) alignment program (having GenBank Accession Nos. P37021 and P09830, respectively, set forth as SEQ ID NOs:28 and 29, respectively).

Figure 7 depicts an alignment of the human 8099 amino acid sequence (SEQ ID NO:2) with the amino acid sequence of the *H. sapiens* facilitative glucose transporter GLUT8 using the CLUSTAL W (1.74) alignment program (having GenBank Accession No. Y02168, set forth as SEQ ID NO:30).

Figures 8A-B depict the cDNA sequence and predicted amino acid sequence of human 46455. The nucleotide sequence corresponds to nucleic acids 1 to 2230 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids 1 to 528 of SEQ ID NO:5. The coding region without the 5' and 3' untranslated regions of the human 46455 gene is shown in SEQ ID NO: 6.

Figure 9 depicts a structural, hydrophobicity, and antigenicity analysis of the human 46455 polypeptide (SEQ ID NO:5).

Figure 10A-C depicts the results of a search which was performed against the HMM database in PFAM.

Figure 11 depicts an alignment of the human 46455 amino acid sequence (SEQ ID NO:5) with the amino acid sequence of *C. elegans* Z92825 using the CLUSTAL W (1.74) alignment program (having GenBank Accession No. Z92825, set forth as SEQ ID NO:31).

Figure 12A-D depicts the nucleotide sequence of the human 54414 cDNA and the corresponding amino acid sequence. The nucleotide sequence corresponds to nucleic acids 1 to 4632 of SEQ ID NO:7. The amino acid sequence corresponds to amino acids 1 to 1118 of SEQ ID NO:8. The coding region without the 5' or 3' untranslated regions of the human 54414 gene is shown in SEQ ID NO:9.

Figure 13 depicts a structural, hydrophobicity, and antigenicity analysis of the human 54414 polypeptide (SEQ ID NO:8). The locations of the 6 transmembrane domains, as well as the pore domain (P), are indicated.

Figure 14 depicts the results of a search in the HMM database, using the amino acid sequence of human 54414.

Figure 15A-B depicts a Clustal W (1.74) multiple sequence alignment of the human 54414 amino acid sequence (54414.prot; SEQ ID NO:8) and the amino acid sequence of the *Rattus norvegicus* Slack potassium channel subunit (AF089730; SEQ ID NO:32; GenBank Accession No. AAC83350). Amino acid identities are indicated by stars. The six transmembrane domains (TM1, TM2, etc.) are boxed. The pore domain, which contains the potassium channel signature sequence motif, is also boxed.

Figure 16A-C depicts the nucleotide sequence of the human 53763 cDNA and the corresponding amino acid sequence. The nucleotide sequence corresponds to nucleic acids 1 to 2847 of SEQ ID NO:10. The amino acid sequence corresponds to amino acids 1 to 638 of SEQ ID NO:11. The coding region without the 5' or 3' untranslated regions of the human 53763 gene is shown in SEQ ID NO:12.

Figure 17 depicts a structural, hydrophobicity, and antigenicity analysis of the human 53763 polypeptide (SEQ ID NO:11). The locations of the 6 transmembrane domains, as well as the pore domain (P), are indicated.

Figure 18A-B depicts the results of a search in the HMM database, using the amino acid sequence of human 53763.

Figure 19 depicts a Clustal W (1.74) sequence alignment of the human 53763 amino acid sequence (Fbh53763pat; SEQ ID NO:11) and the amino acid sequence of the *Rattus norvegicus* voltage-gated potassium channel protein KV3.2 (KSHIIIA) (ratCIKE; SEQ ID NO:33; GenBank Accession No. P22462). Amino acid identities are indicated by stars. The six transmembrane domains (TM1, TM2, etc.) are boxed. The pore domain, which contains the potassium channel signature sequence motif, is also boxed. Plus signs (+) at every third position of the fourth transmembrane domain (TM4), indicate the positively charged residues of the voltage sensor.

Figures 20A-E depicts the cDNA sequence and predicted amino acid sequence of human 67076. The nucleotide sequence corresponds to nucleic acids 1 to 6582 of SEQ ID NO:13. The amino acid sequence corresponds to amino acids 1 to 1129 of SEQ ID NO:14. The coding region without the 5' and 3' untranslated regions of the human 67076 gene is shown in SEQ ID NO:15.

Figure 21 depicts a structural, hydrophobicity, and antigenicity analysis of the human 67076 polypeptide (SEQ ID NO:14).

Figure 22 depicts the results of a search in the HMM database, using the amino acid sequence of human 67076.

Figures 23 depicts a Clustal W (1.74) alignment of the human 67076 amino acid sequence ("Fbh67076FL"; SEQ ID NO:14) with the amino acid sequence of mouse Potential Phospholipid-Transporting ATPase IH (mouseAT1H) (GenBank Accession No. P98197) (SEQ ID NO:34). The transmembrane domains ("TM1", "TM2", etc.), E1-E2 ATPases phosphorylation site ("phosphorylation site"), and phospholipid transporter specific amino acid residues ("phospholipid transport") are boxed.

Figures 24A-E depicts the cDNA sequence and predicted amino acid sequence of human 67102. The nucleotide sequence corresponds to nucleic acids 1 to 6074 of SEQ ID NO:16. The amino acid sequence corresponds to amino acids 1 to 1426 of SEQ ID NO:17. The coding region without the 5' and 3' untranslated regions of the human 67102 gene is shown in SEQ ID NO:18.

Figure 25 depicts a structural, hydrophobicity, and antigenicity analysis of the human 67102 polypeptide (SEQ ID NO:17).

Figure 26A-B depicts the results of a search in the HMM database, using the amino acid sequence of human 67102.

Figures 27A-B depicts a Clustal W (1.74) alignment of the human 67102 amino acid sequence ("Fbh67102FL"; SEQ ID NO:17) with the amino acid sequence of mouse Potential Phospholipid-Transporting ATPase VA (mouseAT5A) (GenBank Accession No. O54827) (SEQ ID NO:35). The transmembrane domains ("TM1", "TM2", etc.), E1-E2 ATPases phosphorylation site ("phosphorylation site"), and phospholipid transporter specific amino acid residues ("phospholipid transport") are boxed.

Figures 28A-E depicts the cDNA sequence and predicted amino acid sequence of human 44181. The nucleotide sequence corresponds to nucleic acids 1 to 7221 of SEQ ID NO:19. The amino acid sequence corresponds to amino acids 1 to 1177 of SEQ ID NO:20. The coding region without the 5' and 3' untranslated regions of the human 44181 gene is shown in SEQ ID NO:21.

Figure 29 depicts a structural, hydrophobicity, and antigenicity analysis of the human 44181 polypeptide (SEQ ID NO:20).

Figure 30A-B depicts the results of a search in the HMM database, using the amino acid sequence of human 44181.

Figures 31A-B depicts a Clustal W (1.74) multiple sequence alignment of the human 44181 amino acid sequence ("Fbh44181"; SEQ ID NO:20) with the amino acid sequence of mouse Potential Phospholipid-Transporting ATPase IH (mouseAT1H) (GenBank Accession No. P98197) (SEQ ID NO:34) and 67076 ("Fbh67076FL"; SEQ ID NO:14). The transmembrane domains ("TM1", "TM2", etc.), E1-E2 ATPases phosphorylation site ("phosphorylation site"), and phospholipid transporter specific amino acid residues ("phospholipid transport") are boxed.

Figures 32A-D depicts the cDNA sequence and predicted amino acid sequence of human 67084FL. The nucleotide sequence corresponds to nucleic acids 1 to 4198 of SEQ ID NO:22. The amino acid sequence corresponds to amino acids 1 to 1084 of SEQ ID NO:23. The coding region without the 5' and 3' untranslated regions of the human 67084FL gene is shown in SEQ ID NO:24.

Figure 33 depicts a structural, hydrophobicity, and antigenicity analysis of the human 67084FL polypeptide (SEQ ID NO:23).

Figures 34A-B depicts the results of a search in the HMM database, using the amino acid sequence of human 67084FL.

Figures 35A-B depicts a Clustal W (1.74) alignment of the human 67084FL amino acid sequence ("Fbh67084FL"; SEQ ID NO:23) with the amino acid sequence of mouse Potential Phospholipid-Transporting ATPase IIV (mouseAT2B) (GenBank Accession No.:P98195) (SEQ ID NO:36). The transmembrane domains ("TM1", "TM2", etc.), E1-E2 ATPases phosphorylation site ("phosphorylation site"), and phospholipid transporter specific amino acid residues ("phospholipid transport") are boxed.

Figures 36A-D depicts the cDNA sequence and predicted amino acid sequence of human 67084alt. The nucleotide sequence corresponds to nucleic acids 1 to 4231 of SEQ ID NO:25. The amino acid sequence corresponds to amino acids 1 to 1095 of SEQ ID NO:26. The coding region without the 5' and 3' untranslated regions of the human 67084alt gene is shown in SEQ ID NO:27.

Figure 37 depicts a structural, hydrophobicity, and antigenicity analysis of the human 67084alt polypeptide (SEQ ID NO:26).

Figures 38A-B depicts the results of a search in the HMM database, using the amino acid sequence of human 67084.

Figures 39A-B depicts a Clustal W (1.74) alignment of the human 67084alt amino acid sequence ("Fbh67084alt"; SEQ ID NO:26) with the amino acid sequence of mouse Potential Phospholipid-Transporting ATPase IIV (mouseAT2B) (GenBank Accession No.:P98195) (SEQ ID NO:36). The transmembrane domains ("TM1", "TM2", etc.), E1-E2 ATPases phosphorylation site ("phosphorylation site"), and phospholipid transporter specific amino acid residues ("phospholipid transport") are boxed.

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of novel sugar transporter family molecules, referred to herein as "8099 and 46455" nucleic acid and polypeptide molecules. These novel molecules are capable of, for example, modulating a transporter mediated activity (*e.g.*, a sugar transporter mediated activity) in a cell, *e.g.*, a liver cell, fat cell, muscle cell, or blood cell, such as an erythrocyte. These novel molecules are capable of transporting molecules, *e.g.*, hexoses such as D-glucose, D-fructose, D-galactose or mannose across biological membranes and, thus, play a role in or function in a variety of cellular processes, *e.g.*, maintenance of sugar homeostasis. Thus the 8099 and 46455 molecules of the present invention provide novel diagnostic targets and therapeutic agents to control 8099 and 46455- associated disorders, as defined herein.

The present invention is also based, at least in part, on the discovery of novel potassium channel family members, referred to herein as "54414 and 53763" nucleic acid and polypeptide molecules. These novel molecules are capable of, for example, modulating PCH mediated activities in a cell, *e.g.*, a neuronal cell. Thus, the 54414 and 53763 molecules of the present invention provide novel diagnostic targets and therapeutic agents to control 54414 or 53763 -associated disorders, as defined herein.

The present invention also is based, at least in part, on the discovery of novel phospholipid transporter family molecules, referred to herein as "67076, 67102, 44181, 67084FL, or 67084alt" nucleic acid and polypeptide molecules. These novel molecules are capable of, for example, transporting phospholipids (*e.g.,* aminophospholipids such as phosphatidylserine and phosphatidylethanolamine, choline phospholipids such as phosphatidylcholine and sphingomyelin, and bile acids) across cellular membranes and, thus, play a role in or function in a variety of cellular processes, *e.g.*, phospholipid transport, absorption, secretion, gene expression, intra- or inter-cellular signaling, and/or cellular proliferation, growth, and/or differentiation. Thus, the 67076, 67102, 44181, 67084FL, and 67084alt molecules of the present invention provide novel diagnostic targets and therapeutic agents to control 67076, 67102, 44181, 67084FL, or 67084alt -associated disorders, as defined herein.

The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin or alternatively, can contain homologues of non-human origin, *e.g.*, rat or mouse proteins. Members of a family can also have common functional characteristics.

8099 and 46455 Molecules of the Invention

The family of 8099 and 46455 polypeptides comprise at least one "transmembrane domain" and at least one, preferably two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve transmembrane domains. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 20-45 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an alpha-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, alanines, valines, phenylalanines, prolines or methionines. Transmembrane domains are described in, for example, Zagotta W.N. *et al,* (1996) *Annual Rev. Neurosci.* 19: 235-263, the contents of which are incorporated herein by reference. A MEMSAT and additional analyses resulted in the identification of twelve transmembrane domains in the amino acid sequence of human 8099 (SEQ ID NO:2) at about residues 32-49, 81-101, 109-130, 138-156, 165-184, 198-217, 279-301, 315-338, 346-364, 463-487, 499-521, and 529-549. A MEMSAT and additional analyses resulted in the identification of twelve transmembrane domains in the amino acid sequence of human 46455 (SEQ ID NO:5) at about residues 58-74, 98-118, 126-145, 165-181, 188-205, 218-238, 273-294, 323-341, 357-377, 386-410, 423-441, and 462-485.

Accordingly, 8099 and 46455 polypeptides having at least 50-60% homology, preferably about 60-70%, more preferably about 70-80%, or about 80-90% homology with at least one, preferably at least two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve transmembrane domains of human 8099 and 46455, respectively are within the scope of the invention.

Another embodiment of the invention features 8099 molecules which contain an N-terminal unique domain. The term "unique N-terminal domain" as used herein, refers to a protein domain of an 8099 protein family member which includes amino acid residues N-terminal to the sixth transmembrane domain, *e.g.,* the GLUT8-like domain in the amino acid

sequence of the 8099 protein. As used herein, a "unique N-terminal domain" refers to a protein domain which is at least about 150-200 amino acid residues in length, preferably at least about 160-190 amino acid residues in length and shares significantly more sequence homology with about residues 1 to 178 of SEQ ID NO:2 than with about residues 1 to 178 of GLUT8.

Accordingly, 8099 polypeptides having at least 50-60% homology, preferably about 60-70%, more preferably about 70-80%, or about 80-90% homology with at least one unique N-terminal domain of human 8099 (*e.g.*, about amino acids 1-178 of SEQ ID NO:2) are within the scope of the invention.

Yet another aspect of the invention features 8099 proteins having an "extended exofacial loop" between transmembrane domains 9 and 10. Preferably, the first amino acid residue of an extended exofacial loop of 8099 is the first residue C-terminal to the amino acid residues of transmembrane domain 9 and the last residue of the exofacial loop is the first residue N-terminal to the amino acid residues of transmembrane domain 10 of 8099. In a preferred embodiment, an extended exofacial loop is at least about 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 85-90, 90-95, 97 or more amino acid residues in length. For example, in one embodiment, an 8099 protein includes an "extended exofacial loop" of about amino acids 365-462 of SEQ ID NO:2 (97 amino acid residues in length).

Accordingly, 8099 polypeptides having at least 50-60% homology, preferably about 60-70%, more preferably about 70-80%, or about 80-90% homology with at least one extended exofacial loop of human 8099 are within the scope of the invention.

In another embodiment, an 8099 and/or 46455 molecule of the present invention is identified based on the presence of at least one "sugar transporter family domain." As used herein, the term "sugar transporter family domain" includes a protein domain having at least about 300-600 amino acid residues and a sugar transporter mediated activity. Preferably, a sugar transporter family domain includes a polypeptide having an amino acid sequence of about 350-550, 400-550, or more preferably, about 411 or 521 amino acid residues and a sugar transporter mediated activity. To identify the presence of a sugar transporter family domain in an 8099 and/or an 46455 protein, and make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein may be searched against a database of known protein domains (*e.g.*, the PFAM HMM database). A PFAM sugar transporter family domain has been assigned the PFAM Accession PF00083. A search was performed against the PFAM HMM database resulting in the identification of a sugar transporter family domain in the amino acid sequence of human 8099 (SEQ ID NO:2) at about residues 43-564 of SEQ ID NO:2. A search was performed against the PFAM HMM database resulting in the identification of a sugar transporter family domain in the amino acid sequence of human 46455 (SEQ ID NO:5) at about residues 58-487 of SEQ ID NO:5.

Preferably a "sugar transporter family domain" has a "sugar transporter mediated activity" as described herein. For example, a sugar transporter family domain may have the ability to bind a monosaccharide (*e.g.*, D-glucose, D-fructose, D-galactose and/or mannose); the ability to transport a monosaccharide (*e.g.*, D-glucose, D-fructose, D-galactose and/or mannose) in a constitutive manner or in response to stimuli (e.g., insulin) across a cell membrane (*e.g.*, a liver cell membrane, fat cell membrane, muscle cell membrane, and/or blood cell membrane, such as an erythrocyte membrane); the ability to function as a neuronal transporter; the ability to mediate trans-epithelial movement; and/or the ability to modulate sugar homeostasis in a cell. Accordingly, identifying the presence of a "sugar transporter family domain" can include isolating a fragment of an 8099 and/or an 46455 molecule (*e.g.*, an 8099 and/or an 46455 polypeptide) and assaying for the ability of the fragment to exhibit one of the aforementioned sugar transporter mediated activities.

A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28:405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.*(1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.*(1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.*(1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.*(1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference.

In a preferred embodiment, the 8099 and/or 46455 molecules of the invention include at least one, preferably two, even more preferably at least three, four, five, six, seven, eight, nine, ten, eleven, or twelve transmembrane domain(s) and/or at least one sugar transporter family domain. In another preferred embodiment, the 8099 molecules of the invention include at least one, preferably two, even more preferably at least three, four, five, six, seven, eight, nine, ten, eleven, or twelve transmembrane domain(s), at least one sugar transporter family domain, at least one unique N-terminal domain, and/or at least one extended exofacial loop.

Isolated polypeptides of the present invention, preferably 8099 or 46455 polypeptides, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or 5 or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:1, 3, 4 or 6. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homology or identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide

sequences which share at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homology or identity and share a common functional activity are defined herein as sufficiently identical.

In a preferred embodiment, an 8099 and/or 46455 polypeptide includes at least one or more of the following domains: a transmembrane domain and/or a sugar transporter family domain, and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous or identical to the amino acid sequence of SEQ ID NO:2 or 5, or the amino acid sequences encoded by the DNA inserts of the plasmids deposited with ATCC as Accession Numbers _____ and/or _____. In yet another preferred embodiment, an 8099 and/or an 46455 polypeptide includes at least one or more of the following domains: a transmembrane domain and/or a sugar transporter family domain, and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:6. In another preferred embodiment, an 8099 and/or an 46455 polypeptide includes at least one or more of the following domains: a transmembrane domain and/or a sugar transporter family domain, and has an 8099 and/or an 46455 activity.

As used interchangeably herein, an "8099 activity", "46455 activity", "biological activity of 8099", "biological activity of 46455", "functional activity of 8099" or "functional activity of 46455" refers to an activity exerted by an 8099 and/or 46455 polypeptide or nucleic acid molecule on an 8099 and/or 46455 responsive cell or tissue, or on an 8099 and/or 46455 polypeptide substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, an 8099 and/or 46455 activity is a direct activity, such as an association with an 8099- and/or 46455-target molecule. As used herein, a "substrate," "target molecule," or "binding partner" is a molecule with which an 8099 and/or 46455 polypeptide binds or interacts in nature, such that 8099- and/or 46455-mediated function is achieved. An 8099 and/or 46455 target molecule can be a non- 8099 and/or a non-46455 molecule or an 8099 and/or 46455 polypeptide or polypeptide of the present invention. In an exemplary embodiment, an 8099 and/or 46455 target molecule is an 8099 and/or 46455 ligand, *e.g.*, a sugar transporter ligand such D-glucose, D-fructose, D-galactose, and/or mannose. Alternatively, an 8099 and/or 46455 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the 8099 and/or 46455 polypeptide with an 8099 and/or 46455 ligand. The biological activities of 8099 and/or 46455 are described herein. For example, the 8099 and/or 46455 polypeptides of the present invention can have one or more of the following activities: (1) bind a monosaccharide, *e.g.*, D-glucose, D-fructose, D-galactose, and/or mannose, (2) transport monosaccharides across a cell membrane, (3) influence insulin and/or glucagon secretion, (4) maintain sugar homeostasis in a cell, (5) function as a neuronal transporter, and (6) mediate trans-epithelial movement in

a cell. Moreover, in a preferred embodiment, 8099 and/or 46455 molecules of the present invention, 8099 and/or 46455 antibodies, 8099 and/or 46455 modulators are useful in at least one of the following: (1) modulation of insulin sensitivity; (2) modulation of blood sugar levels; (3) treatment of blood sugar level disorders (*e.g.*, diabetes); and/or (4) modulation of insulin resistance.

The nucleotide sequence of the isolated human 8099 and 46455 cDNAs and the predicted amino acid sequences of the human 8099 and 46455 polypeptides are shown in Figures 1 and 8 and in SEQ ID NOs:1 and 2, and SEQ ID NOs:4 and 5, respectively. Plasmids containing the nucleotide sequences encoding human 8099 or 46455 were deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Numbers _____ or _____. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112.

The human 8099 gene, which is approximately 2725 nucleotides in length, encodes a polypeptide which is approximately 617 amino acid residues in length. The human 46455 gene, which is approximately 2230 nucleotides in length, encodes a polypeptide which is approximately 528 amino acid residues in length.

54414 and 53763 Molecules of the Invention

The family of 54414 and 53763 proteins of the present invention comprises at least one transmembrane domain, preferably at least 2 or 3 transmembrane domains, more preferably 4 or 5 transmembrane domains, and most preferably, 6 transmembrane domains. Amino acid residues 64-83, 104-127, 135-153, 161-173, 199-217, and 257-274 of the human 54414 protein (SEQ ID NO:8) are predicted to comprise transmembrane domains. Amino acid residues 230-248, 287-303, 314-335, 346-368, 382-402, and 451-473 of the human 53763 protein (SEQ ID NO:11) are predicted to comprise transmembrane domains.

In another embodiment, members of the 54414 and 53763 family of proteins include at least one "ion transport protein domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "ion transport protein domain" includes a protein domain having at least about 150-310 amino acid residues and a bit score of at least 200 when compared against an ion transport protein domain Hidden Markov Model (HMM), *e.g.*, PFAM Accession Number PF00520. Preferably, an ion transport protein domain includes a protein domain having an amino acid sequence of about 170-290, 190-270, 210-250, or more preferably about 173 or 191 amino acid residues. To identify the presence of an ion transport protein domain in a 54414 or 53763 protein, and make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is

searched against a database of known protein motifs and/or domains (*e.g.*, the HMM database). The ion transport protein domain (HMM) has been assigned the PFAM Accession number PF00520. A search was performed against the HMM database resulting in the identification of an ion transport protein domain in the amino acid sequence of human 54414 at about residues 104-277 of SEQ ID NO:8 and in the amino acid sequence of human 53763 about residues 281-472 of SEQ ID NO:11.

Preferably an ion transport protein domain is at least about 150-310 amino acid residues and has an "ion transport protein domain activity", for example, the ability to interact with a 54414 or 53763 substrate or target molecule (*e.g.*, a potassium ion) and/or to regulate 54414 or 53763 activity. Accordingly, identifying the presence of an "ion transport protein domain" can include isolating a fragment of a 54414 or 53763 molecule (*e.g.*, a 54414 or 53763 polypeptide) and assaying for the ability of the fragment to exhibit one of the aforementioned ion transport protein domain activities.

In another embodiment, members of the 54414 and 53763 family of proteins include at least one "K^+ channel tetramerisation domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "K^+ channel tetramerisation domain" includes a protein domain having at least about 70-230 amino acid residues and a bit score of at least 80 when compared against a K^+ channel tetramerisation domain Hidden Markov Model (HMM), *e.g.*, PFAM Accession Number PF02214. Preferably, a K^+ channel tetramerisation domain includes a protein domain having an amino acid sequence of about 90-210, 110-190, 130-170, or more preferably about 149 amino acid residues, and a bit score of at least 100, 120, 140, or more preferably, 156.7. To identify the presence of a K^+ channel tetramerisation domain in a 54414 or 53763 protein, and make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein is searched against a database of known protein motifs and/or domains (*e.g.*, the HMM database). The K^+ channel tetramerisation domain (HMM) has been assigned the PFAM Accession number PF02214. A search was performed against the HMM database resulting in the identification of a K^+ channel tetramerisation domain in the amino acid sequence of human 53763 at about residues 8-156 of SEQ ID NO:11.

Preferably a K^+ channel tetramerisation domain is at least about 70-230 amino acid residues and has an "K^+ channel tetramerisation domain activity", for example, the ability to interact with one or more potassium channel subunits *(e.g.,* 54414 or 53763 molecules, or non-54414 or 53763 potassium channel subunits), the ability to regulate assembly of a 54414 or 53763 molecule into a potassium channel tetramer, and/or to regulate 54414 or 53763 activity. Accordingly, identifying the presence of an "K^+ channel tetramerisation domain" can include isolating a fragment of a 54414 or 53763 molecule (*e.g.*, a 54414 or 53763 polypeptide) and assaying for the ability of the fragment to exhibit one of the aforementioned K^+ channel tetramerisation domain activities.

In another embodiment, a 54414 or 53763 protein of the present invention is identified based on the presence of an "ATP/GTP-binding sit motif A (P-loop) motif", referred to alternatively herein as a "P-loop motif", in the protein or corresponding nucleic acid molecule. Preferably, a P-loop motif includes a protein motif which is about 4-15, 5-13, 6-11, 7-9, or preferably about 8 amino acid residues. The P-loop motif functions in binding ATP and/or GTP via interaction with the phosphate groups of the nucleotide and has been assigned Prosite™ Accession Number PS00017. To identify the presence of a P-loop motif in a 54414 or 53763 protein, and to make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein may be searched against a database of known protein domains or motifs (*e.g.*, the Prosite™ database) using the default parameters (available at the ProSite website). A search was performed against the ProSite database resulting in the identification of a P-loop motif in the amino acid sequence of human 54414 (SEQ ID NO:8) at about residues 1007-1014.

In another embodiment, a 54414 or 53763 protein of the present invention is identified based on the presence of a "pore domain", alternatively referred to herein as a "P-region domain", in the protein or corresponding nucleic acid molecule. As used interchangeably herein, the terms "pore domain" and "P-region domain" include a protein domain having about 10-30, 12-28, 13-25, 14-24, 15-23, or preferably about 16-22 amino acid residues, which is involved in lining the potassium channel pore. A pore domain is typically found between transmembrane domains of potassium channels and is believed to be a major determinant of ion selectivity in potassium channels. Preferably, a pore domain includes a potassium channel signature motif, as defined herein. Pore domains are described in, for example, Warmke et al. (1991) *Science* 252:1560-1562; Zagotta W.N. et al. (1996) *Annu. Rev. Neurosci.* 19:235-63; Pongs, O. (1993) *J. Membr. Biol.* 136:1-8; Heginbotham et al. (1994) *Biophys. J.* 66:1061-1067; Mackinnon, R. (1995) *Neuron* 14:889-892; and Pascual et al. (1995) *Neuron* 14:1055-1063), the contents of which are incorporated herein by reference. A pore domain was identified in the amino acid sequence of human 54414 at about residues 229-250 of SEQ ID NO:8. A pore domain was identified in the amino acid sequence of human 53763 at about residues 426-441 of SEQ ID NO:11.

In a further embodiment, a 54414 or 53763 protein of the present invention is identified based on the presence of a "potassium channel signature sequence motif" in the protein or corresponding nucleic acid molecule. As used herein, the term "potassium channel signature sequence motif" includes a protein motif which is diagnostic for potassium channels. Preferably, a potassium channel signature sequence motif has the consensus sequence T-X-X-T-X-G-hydrophobic-G (see Joiner, W. J. et al. (1998) *Nat. Neurosci.* 1:462-469 and references cited therein), wherein "X" indicates any amino acid residue, and "hydrophobic" indicates any hydrophobic amino acid residue. Preferably, a potassium channel signature sequence motif is included within a pore domain and includes

at least 1, 2, 3, 4, 5, 6, 7, or more preferably, 8 amino acid residues that match the consensus sequence for a potassium channel signature sequence motif. A potassium channel signature sequence motif was identified in the amino acid sequence of human 54414 at about residues 239-246 of SEQ ID NO:8. A potassium channel signature sequence motif was identified in the amino acid sequence of human 53763 at about residues 436-441 of SEQ ID NO:11.

In still another embodiment, a 54414 or 53763 protein of the present invention is identified based on the presence of a "voltage sensor motif", alternatively referred to simply as a "voltage sensor", in the protein or the corresponding nucleic acid molecule. As used interchangeably herein, the terms "voltage sensor motif" and "voltage sensor" include a protein motif having about 10-30, 11-26, 12-24, 13-22, 14-20, 15-18, or more preferably 16 amino acid residues, which is involved in sensing voltage differences between the two sides of the plasma membrane of a cell. Preferably, a voltage sensor motif includes at least 1, 2, 3, 4, 5, or more preferably, 6 positively charged amino acid residues, which are preferably spaced apart by at least 1, or preferably 2, non-positively charged amino acid residues. Preferably, a voltage sensor motif is included within and/or overlaps with a transmembrane domain, more preferably the fourth transmembrane, of the 54414 or 53763 protein in which it is found. A voltage sensor motif was identified in the amino acid sequence of human 53763 at about residues 348-363 of SEQ ID NO:8. The positively charged amino acid residues of the human 53763 voltage sensor were identified at about residues 348, 351, 354, 357, 360, and 363 of SEQ ID NO:8. No voltage sensor was identified in human 54414.

Isolated proteins of the present invention, preferably 54414 or 53763 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:11, or are encoded by a nucleotide sequence sufficiently homologous to SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:12. Amino acid or nucleotide sequences which share common structural domains having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homology or identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homology or identity and share a common functional activity are defined herein as sufficiently homologous.

In a preferred embodiment, a 54414 or 53763 protein includes at least one or more of the following domains or motifs: a transmembrane domain, an ion transport protein domain, a K^+ channel tetramerisation domain, a P-loop motif, a pore domain, a potassium channel signature sequence motif, and/or a voltage sensor motif. and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous or identical to the amino acid sequence of SEQ

ID NO:8 or 11, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____ or _____. In yet another preferred embodiment, a 54414 or 53763 protein includes at least one or more of the following domains or motifs: a transmembrane domain, an ion transport protein domain, a K^+ channel tetramerisation domain, a P-loop motif, a pore domain, a potassium channel signature sequence motif, and/or a voltage sensor motif, and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:7, 9, 10, or 12. In another preferred embodiment, a 54414 or 53763 protein includes at least one or more of the following domains or motifs: a transmembrane domain, an ion transport protein domain, a K^+ channel tetramerisation domain, a P-loop motif, a pore domain, a potassium channel signature sequence motif, and/or a voltage sensor motif, and has a 54414 or 53763 activity.

As used interchangeably herein, a "54414 or 53763 activity", "biological activity of 54414 or 53763" or "functional activity of 54414 or 53763", includes an activity exerted or mediated by a 54414 or 53763 protein, polypeptide or nucleic acid molecule when expressed in a cell or on a membrane, as determined *in vivo* or *in vitro*, according to standard techniques. In one embodiment, a 54414 or 53763 activity is a direct activity, such as transport of a 54414 or 53763 substrate (*e.g.*, a potassium ion). In another embodiment, a 54414 or 53763 activity is an indirect activity mediated, for example, by interaction of a 54414 or 53763 molecule with a 54414 or 53763 target molecule or binding partner. As used herein, a "target molecule" or "binding partner" is a molecule with which a 54414 or 53763 protein binds or interacts in nature, such that function of the target molecule or binding partner is modulated. In an exemplary embodiment, a 54414 or 53763 target molecule or binding partner is a 54414 or 53763 polypeptide or a non-54414 or 53763 potassium channel subunit.

In a preferred embodiment, a 54414 or 53763 activity is at least one of the following activities: (i) interaction with a 54414 or 53763 substrate (*e.g.*, a potassium ion or a cyclic nucleotide); (ii) conductance or transport of a 54414 or 53763 substrate across a cellular membrane; (iii) interaction with a second protein (*e.g.*, a second 54414 or 53763 subunit or a non-54414 or 53763 potassium channel subunit); (iv) modulation *(e.g.*, maintenance and/or rectification) of membrane potentials; (v) regulation of target molecule availability or activity; (vi) modulation of intra- or intercellular signaling and/or gene transcription (*e.g.*, either directly or indirectly); (viii) generation of outwardly rectifying currents; (viii) modulation of membrane excitability; (ix) modulation of the release of neurotransmitters; (x) regulation of contractility *(e.g.,* of smooth muscle cells), secretion, and/or synaptic transmission; and/or (xi) modulation of processes which underlie learning and memory.

Preferred activities of 54414 further include at least one of the following activities: (i) interaction with maxi-K potassium channels (*i.e.*, large conductance channels, in particular *Slo*); (ii) modulation of maxi-K potassium channel activity (e.g., *Slo*-mediated activities); (iii) generation of intermediate conductance channels; and/or (iv) regulation of contractility (*e.g.*, of smooth muscle cells), secretion, and/or synaptic transmission, in particular, via modulation of *Slo*.

Preferred activities of 53763 further include at least one of the following activities: (i) interaction with Shaker (Sh) potassium channels and/or channel subunits; (ii) modulation of Shaker (Sh) potassium channel activity (*e.g.*, termination of prolonged membrane depolarization; (iii) modulation of high voltage activating channel activity and/or inactivating channel activity, and the like.

The nucleotide sequence of the isolated human 54414 cDNA and the predicted amino acid sequence encoded by the 54414 cDNA are shown in Figures 12A-C and in SEQ ID NOs:7 and 8, respectively. A plasmid containing the human 54414 cDNA was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit were made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The human 54414 gene, which is approximately 4632 nucleotides in length, encodes a protein having a molecular weight of approximately 123 kD and which is approximately 1118 amino acid residues in length.

The nucleotide sequence of the isolated human 53763 cDNA and the predicted amino acid sequence encoded by the 53763 cDNA are shown in Figures 16A-C and in SEQ ID NOs:10 and 11, respectively. A plasmid containing the human 53763 cDNA was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit were made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The human 53763 gene, which is approximately 2847 nucleotides in length, encodes a protein having a molecular weight of approximately 70.2 kD and which is approximately 638 amino acid residues in length.

67076, 67102, 44181, 67084FL, and 67084alt Molecules of the Invention

The 67076, 67102, 44181, 67084FL, and 67084alt polypeptides comprise at least one "transmembrane domain" and preferably eight, nine, or ten transmembrane domains. A MEMSAT analysis and a structural, hydrophobicity, and antigenicity analysis also resulted in the identification of ten transmembrane domains in the amino acid sequence of human 67076 (SEQ ID NO:14) at about residues 57-77, 84-105, 292-313, 345-365, 863-883, 905-926, 956-977, 989-1009, 1021-1041, and 1060-1087. A MEMSAT analysis and a structural, hydrophobicity, and antigenicity analysis resulted in the identification of ten transmembrane domains in the amino acid sequence of human 67102 (SEQ ID NO:17) at about residues 98-115, 122-140, 322-344, 366-390, 582-601, 752-770, 1145-1166, 1225-1246, 1253-1276, and 1298-1317. A MEMSAT analysis and a structural, hydrophobicity, and antigenicity analysis resulted in the identification of ten transmembrane domains in the amino acid sequence of human 44181 (SEQ ID NO:20) at about residues 56-72, 87-103, 290-311, 343-363, 878-898, 911-931, 961-982, 995-1015, 1027-1047, and 1062-1086. A MEMSAT analysis and a structural, hydrophobicity, and antigenicity analysis resulted in the identification of ten transmembrane domains in the amino acid sequence of human 67084FL (SEQ ID NO:23) at about residues 104-120, 124-144, 331-350, 357-374, 887-903, 912-931, 961-983, 990-1008, 1015-1035, and 1043-1067. A MEMSAT analysis and a structural, hydrophobicity, and antigenicity analysis resulted in the identification of ten transmembrane domains in the amino acid sequence of human 67084alt (SEQ ID NO:26) at about residues 104-120, 124-144, 331-350, 357-379, 887-903, 912-931, 961-983, 990-1008, 1015-1035, and 1054-1078.

The family of 67076, 67102, 44181, 67084FL, or 67084alt proteins of the present invention also comprises at least one "extramembrane domain" in the protein or corresponding nucleic acid molecule. As used herein, an "extramembrane domain" includes a domain having greater than 20 amino acid residues that is found between transmembrane domains, preferably on the cytoplasmic side of the plasma membrane, and does not span or traverse the plasma membrane. An extramembrane domain preferably includes at least one, two, three, four or more motifs or consensus sequences characteristic of P-type ATPases, *i.e.*, includes one, two, three, four, or more "P-type ATPase consensus sequences or motifs". As used herein, the phrase "P-type ATPase consensus sequences or motifs" includes any consensus sequence or motif known in the art to be characteristic of P-type ATPases, including, but not limited to, the P-type ATPase sequence 1 motif (as defined herein), the P-type ATPase sequence 2 motif (as defined herein), the P-type ATPase sequence 3 motif (as defined herein), and the E1-E2 ATPases phosphorylation site (as defined herein).

In one embodiment, the family of 67076, 67102, 44181, 67084FL, or 67084alt proteins of the present invention comprises at least one "N-terminal" large extramembrane domain in the protein or corresponding nucleic acid molecule. As used herein, an "N-

terminal" large extramembrane domain is found in the N-terminal 1/3rd of the protein, preferably between the second and third transmembrane domains of a 67076, 67102, 44181, 67084FL, or 67084alt protein and includes about 60-300, 80-280, 100-260, 120-240, 140-220, 160-200, or preferably, 180, 185, or 186 amino acid residues. In a preferred embodiment, an N-terminal large extramembrane domain includes at least one P-type ATPase sequence 1 motif (as described herein). An N-terminal large extramembrane domain was identified in the amino acid sequence of human 67076 at about residues 106-291 of SEQ ID NO:14. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 67102 at about residues 141-321 of SEQ ID NO:17. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 44181 at about residues 104-289 of SEQ ID NO:20. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 67084FL at about residues 145-330 of SEQ ID NO:23. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 67087alt at about residues 145-330 of SEQ ID NO:26.

The family of 67076, 67102, 44181, 67084FL, or 67084alt proteins of the present invention also comprises at least one "C-terminal" large extramembrane domain in the protein or corresponding nucleic acid molecule. As used herein, a "C-terminal" large extramembrane domain is found in the C-terminal 2/3rds of the protein, preferably between the fourth and fifth transmembrane domains of a 67076, 67102, 44181, 67084FL, or 67084alt protein and includes about 150-1000, 300-900, 370-850, 400-820, 430-790, 460-760, 430-730, 460-700, 430-670, 460-640, 430-610, 490-580, 510-550, or preferably, 190, 506, or 523 amino acid residues. In a preferred embodiment, a C-terminal large extramembrane domain includes at least one or more of the following motifs: a P-type ATPase sequence 2 motif (as described herein), a P-type ATPase sequence 3 motif (as defined herein), and/or an E1-E2 ATPases phosphorylation site (as defined herein). A C-terminal large extramembrane domain was identified in the amino acid sequence of human 67076 at about residues 366-862 of SEQ ID NO:14. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 67102 at about residues 391-581 of SEQ ID NO:17. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 44181 at about residues 364-877 of SEQ ID NO:20. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 67084FL at about residues 380-886 of SEQ ID NO:23. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 67084alt at about residues 380-886 of SEQ ID NO:26.

In another embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein or 67076, 67102, 44181, 67084FL, or 67084alt extramembrane domain is characterized by at least one "P-type ATPase sequence 1 motif" in the protein or corresponding nucleic acid sequence. As used herein, a "P-type ATPase sequence 1 motif" is a conserved sequence

motif diagnostic for P-type ATPases (Tang, X. *et al.* (1996) *Science* 272:1495-1497; Fagan, M. J. and Saier, M. H. (1994) *J. Mol. Evol.* 38:57). Amino acid residues of the P-type ATPase sequence 1 motif are involved in the coupling of ATP hydrolysis with transport (*e.g.,* transport of phospholipids). The consensus sequence for a P-type ATPase sequence 1 motif is [DNS]-[QENR]-[SA]-[LIVSAN]-[LIV]-[TSN]-G-E-[SN] (SEQ ID NO:37). The use of amino acids in brackets indicates that the amino acid at the indicated position may be any one of the amino acids within the brackets, *e.g.*, [SA] indicates any of one of either S (serine) or A (alanine). In a preferred embodiment, a P-type ATPase sequence 1 motif is contained within an N-terminal large extramembrane domain. In another preferred embodiment, a P-type ATPase sequence 1 motif in the 67076, 67102, 44181, 67084FL, or 67084alt proteins of the present invention has at least 1, 2, 3, or preferably 4 amino acid resides which match the consensus sequence for a P-type ATPase sequence 1 motif. A P-type ATPase sequence 1 motif was identified in the amino acid sequence of human 67076 at about residues 173-181 of SEQ ID NO:14. A P-type ATPase sequence 1 motif was identified in the amino acid sequence of human 67102 at about residues 208-216 of SEQ ID NO:17. A P-type ATPase sequence 1 motif was identified in the amino acid sequence of human 44181 at about residues 173-181 of SEQ ID NO:20. A P-type ATPase sequence 1 motif was identified in the amino acid sequence of human 67084FL at about residues 213-221 of SEQ ID NO:23. A P-type ATPase sequence 1 motif was identified in the amino acid sequence of human 67084alt at about residues 213-221 of SEQ ID NO:26.

In another embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein or 67076, 67102, 44181, 67084FL, or 67084alt extramembrane domain is characterized by at least one "P-type ATPase sequence 2 motif" in the protein or corresponding nucleic acid sequence. As used herein, a "P-type ATPase sequence 2 motif" is a conserved sequence motif diagnostic for P-type ATPases (Tang, X. et al. (1996) *Science* 272:1495-1497; Fagan, M. J. and Saier, M. H. (1994) *J. Mol. Evol.* 38:57). Preferably, a P-type ATPase sequence 2 motif overlaps with and/or includes an E1-E2 ATPases phosphorylation site (as defined herein). The consensus sequence for a P-type ATPase sequence 2 motif is [LIV]-[CAML]-[STFL]-D-K-T-G-T-[LI]-T (SEQ ID NO:38). The use of amino acids in brackets indicates that the amino acid at the indicated position may be any one of the amino acids within the brackets, *e.g.*, [LI] indicates any of one of either L (leucine) or I (isoleucine). In a preferred embodiment, a P-type ATPase sequence 2 motif is contained within a C-terminal large extramembrane domain. In another preferred embodiment, a P-type ATPase sequence 2 motif in the 67076, 67102, 44181, 67084FL, or 67084alt proteins of the present invention has at least 1, 2, 3, 4, 5, 6, 7, 8, or more preferably 9 amino acid resides which match the consensus sequence for a P-type ATPase sequence 2 motif. A P-type ATPase sequence 2 motif was identified in the amino acid sequence of human 67076 at about residues 406-415 of SEQ ID NO:14. A P-type ATPase sequence 2 motif was identified in the amino acid

sequence of human 67102 at about residues 435-444 of SEQ ID NO:17. A P-type ATPase sequence 2 motif was identified in the amino acid sequence of human 44181 at about residues 404-413 of SEQ ID NO:20. A P-type ATPase sequence 2 motif was identified in the amino acid sequence of human 67084FL at about residues 413-422 of SEQ ID NO:23. A P-type ATPase sequence 2 motif was identified in the amino acid sequence of human 67084alt at about residues 413-422 of SEQ ID NO:26.

In yet another embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein or 67076, 67102, 44181, 67084FL, or 67084alt extramembrane domain is characterized by at least one "P-type ATPase sequence 3 motif" in the protein or corresponding nucleic acid sequence. As used herein, a "P-type ATPase sequence 3 motif" is a conserved sequence motif diagnostic for P-type ATPases (Tang, X. et al. (1996) *Science* 272:1495-1497; Fagan, M. J. and Saier, M. H. (1994) *J. Mol. Evol.* 38:57). Amino acid residues of the P-type ATPase sequence 3 motif are involved in ATP binding. The consensus sequence for a P-type ATPase sequence 3 motif is [TIV]-G-D-G-X-N-D-[ASG]-P-[ASV]-L (SEQ ID NO:39). X indicates that the amino acid at the indicated position may be any amino acid (*i.e.*, is not conserved). The use of amino acids in brackets indicates that the amino acid at the indicated position may be any one of the amino acids within the brackets, *e.g.*, [TIV] indicates any of one of either T (threonine), I (isoleucine), or V (valine). In a preferred embodiment, a P-type ATPase sequence 3 motif is contained within a C-terminal large extramembrane domain. In another preferred embodiment, a P-type ATPase sequence 3 motif in the 67076, 67102, 44181, 67084FL, or 67084alt proteins of the present invention has at least 1, 2, 3, 4, 5, 6, or more preferably 7 amino acid resides (including the amino acid at the position indicated by "X") which match the consensus sequence for a P-type ATPase sequence 3 motif. A P-type ATPase sequence 3 motif was identified in the amino acid sequence of human 67076 at about residues 813-824 of SEQ ID NO:14. A P-type ATPase sequence 3 motif was identified in the amino acid sequence of human 67102 at about residues 1054-1064 of SEQ ID NO:17. A P-type ATPase sequence 3 motif was identified in the amino acid sequence of human 44181 at about residues 819-829 of SEQ ID NO:20. A P-type ATPase sequence 3 motif was identified in the amino acid sequence of human 67084FL at about residues 820-830 of SEQ ID NO:23. A P-type ATPase sequence 3 motif was identified in the amino acid sequence of human 67084alt at about residues 820-830 of SEQ ID NO:26.

In another embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein of the present invention is identified based on the presence of an "E1-E2 ATPases phosphorylation site" (alternatively referred to simply as a "phosphorylation site") in the protein or corresponding nucleic acid molecule. An E1-E2 ATPases phosphorylation site functions in accepting a phosphate moiety and has the amino acid sequence DKTGT (amino acid residues 4-8 of SEQ ID NO:38), and can be included within the E1-E2 ATPase

phosphorylation site consensus sequence: D-K-T-G-T-[LIVM]-[TI] (SEQ ID NO:41), wherein D is phosphorylated. The use of amino acids in brackets indicates that the amino acid at the indicated position may be any one of the amino acids within the brackets, *e.g.*, [TI] indicates any of one of either T (threonine) or I (isoleucine). The E1-E2 ATPases phosphorylation site consensus sequence has been assigned ProSite Accession Number PS00154. To identify the presence of an E1-E2 ATPases phosphorylation site consensus sequence in a 67076, 67102, 44181, 67084FL, or 67084alt protein, and to make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein may be searched against a database of known protein motifs (*e.g.*, the ProSite database) using the default parameters (available at the Prosite website). A search was performed against the ProSite database resulting in the identification of an E1-E2 ATPases phosphorylation site consensus sequence in the amino acid sequence of human 67076 (SEQ ID NO:14) at about residues 409-415. A search was performed against the ProSite database resulting in the identification of an E1-E2 ATPases phosphorylation site consensus sequence in the amino acid sequence of human 67102 (SEQ ID NO:17) at about residues 438-444. A search was performed against the ProSite database resulting in the identification of an E1-E2 ATPases phosphorylation site consensus sequence in the amino acid sequence of human 44181 (SEQ ID NO:20) at about residues 407-413. A search was performed against the ProSite database resulting in the identification of an E1-E2 ATPases phosphorylation site consensus sequence in the amino acid sequence of human 67084FL (SEQ ID NO:23) at about residues 416-422. A search was performed against the ProSite database resulting in the identification of an E1-E2 ATPases phosphorylation site consensus sequence in the amino acid sequence of human 67084alt (SEQ ID NO:26) at about residues 416-422.

Preferably an E1-E2 ATPases phosphorylation site has a "phosphorylation site activity," for example, the ability to be phosphorylated; to be dephosphorylated; to regulate the E1-E2 conformational change of the phospholipid transporter in which it is contained; to regulate transport of phospholipids (*e.g.*, aminophospholipids such as phosphatidylserine and phosphatidylethanolamine, choline phospholipids such as phosphatidylcholine and sphingomyelin, and bile acids) across a cellular membrane by the 67076, 67102, 44181, 67084FL, or 67084alt protein in which it is contained; and/or to regulate the activity (as defined herein) of the 67076, 67102, 44181, 67084FL, or 67084alt protein in which it is contained. Accordingly, identifying the presence of an "E1-E2 ATPases phosphorylation site" can include isolating a fragment of a 67076, 67102, 44181, 67084FL, or 67084alt molecule (*e.g.*, a 67076, 67102, 44181, 67084FL, or 67084alt polypeptide) and assaying for the ability of the fragment to exhibit one of the aforementioned phosphorylation site activities.

In another embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein of the present invention may also be identified based on its ability to adopt an E1 conformation or

an E2 conformation. As used herein, an "E1 conformation" of a 67076, 67102, 44181, 67084FL, or 67084alt protein includes a 3-dimensional conformation of a 67076, 67102, 44181, 67084FL, or 67084alt protein which does not exhibit 67076, 67102, 44181, 67084FL, or 67084alt activity (*e.g.*, the ability to transport phospholipids), as defined herein. An E1 conformation of a 67076, 67102, 44181, 67084FL, or 67084alt protein usually occurs when the 67076, 67102, 44181, 67084FL, or 67084alt protein is unphosphorylated. As used herein, an "E2 conformation" of a 67076, 67102, 44181, 67084FL, or 67084alt protein includes a 3-dimensional conformation of a 67076, 67102, 44181, 67084FL, or 67084alt protein which exhibits 67076, 67102, 44181, 67084FL, or 67084alt activity (*e.g.*, the ability to transport phospholipids), as defined herein. An E2 conformation of a 67076, 67102, 44181, 67084FL, or 67084alt protein usually occurs when the 67076, 67102, 44181, 67084FL, or 67084alt protein is phosphorylated.

In still another embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein of the present invention is identified based on the presence of "phospholipid transporter specific" amino acid residues. As used herein, "phospholipid transporter specific" amino acid residues are amino acid residues specific to the class of phospholipid transporting P-type ATPases (as defined in Tang, X. et al. (1996) *Science* 272:1495-1497). Phospholipid transporter specific amino acid residues are not found in those P-type ATPases which transport molecules which are not phospholipids (*e.g.*, cations). For example, phospholipid transporter specific amino acid residues are found at the first, second, and fifth positions of the P-type ATPase sequence 1 motif. In phospholipid transporting P-type ATPases, the first position of the P-type ATPase sequence 1 motif is preferably E (glutamic acid), the second position is preferably T (threonine), and the fifth position is preferably L (leucine). A phospholipid transporter specific amino acid residue is further found at the second position of the P-type ATPase sequence 2 motif. In phospholipid transporting P-type ATPases, the second position of the P-type ATPase sequence 2 motif is preferably F (phenylalanine). Phospholipid transporter specific amino acid residues are still further found at the first, tenth, and eleventh positions of the P-type ATPase sequence 3 motif. In phospholipid transporting P-type ATPases, the first position of the P-type ATPase sequence 3 motif is preferably I (isoleucine), the tenth position is preferably M (methionine), and the eleventh position is preferably I (isoleucine).

Phospholipid transporter specific amino acid residues were identified in the amino acid sequence of human 67076 (SEQ ID NO:14) at about residues 174 and 177 (within the P-type ATPase sequence 1 motif), at about residue 407 (within the P-type ATPase sequence 2 motif), and at about residues 813, 823, and 824 (within the P-type ATPase sequence 3 motif).

Phospholipid transporter specific amino acid residues were identified in the amino acid sequence of human 67102 (SEQ ID NO:17) at about residues 208, 209, and 212 (within

the P-type ATPase sequence 1 motif), at about residue 436 (within the P-type ATPase sequence 2 motif), and at about residues 1054, 1063, and 1064 (within the P-type ATPase sequence 3 motif).

Phospholipid transporter specific amino acid residues were identified in the amino acid sequence of human 44181 (SEQ ID NO:20) at about residues 174 and 177 (within the P-type ATPase sequence 1 motif), at about residue 405 (within the P-type ATPase sequence 2 motif), and at about residues 819, 828, and 829 (within the P-type ATPase sequence 3 motif).

Phospholipid transporter specific amino acid residues were identified in the amino acid sequence of human 67084FL (SEQ ID NO:23) at about residues 214 and 217 (within the P-type ATPase sequence 1 motif) and at about residues 820, 829, and 830 (within the P-type ATPase sequence 3 motif).

Phospholipid transporter specific amino acid residues were identified in the amino acid sequence of human 67084alt (SEQ ID NO:26) at about residues 214 and 217 (within the P-type ATPase sequence 1 motif), and at about residues 820, 829, and 830 (within the P-type ATPase sequence 3 motif).

Isolated polypeptides of the present invention, preferably 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26 or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:27. For example, amino acid or nucleotide sequences which share common structural domains having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homology or identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences which share at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homology or identity and share a common functional activity are defined herein as sufficiently identical.

In a preferred embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein includes at least one or more of the following domains, sites, or motifs: a transmembrane domain, an N-terminal large extramembrane domain, a C-terminal large extramembrane domain, an E1-E2 ATPases phosphorylation site, a P-type ATPase sequence 1 motif, a P-type ATPase sequence 2 motif, a P-type ATPase sequence 3 motif, and/or one or more phospholipid transporter specific amino acid resides, and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous or identical to the amino acid sequence of SEQ ID NO:14, SEQ

ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____. In yet another preferred embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein includes at least one or more of the following domains, sites, or motifs: a transmembrane domain, an N-terminal large extramembrane domain, a C-terminal large extramembrane domain, an E1-E2 ATPases phosphorylation site, a P-type ATPase sequence 1 motif, a P-type ATPase sequence 2 motif, a P-type ATPase sequence 3 motif, and/or one or more phospholipid transporter specific amino acid resides, and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:27. In another preferred embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt protein includes at least one or more of the following domains, sites, or motifs: a transmembrane domain, an N-terminal large extramembrane domain, a C-terminal large extramembrane domain, an E1-E2 ATPases phosphorylation site, a P-type ATPase sequence 1 motif, a P-type ATPase sequence 2 motif, a P-type ATPase sequence 3 motif, and/or one or more phospholipid transporter specific amino acid resides, and has a 67076, 67102, 44181, 67084FL, or 67084alt activity.

As used interchangeably herein, a "phospholipid transporter activity" or a "67076, 67102, 44181, 67084FL, or 67084alt activity" includes an activity exerted or mediated by a 67076, 67102, 44181, 67084FL, or 67084alt protein, polypeptide or nucleic acid molecule on a 67076, 67102, 44181, 67084FL, or 67084alt responsive cell or on a 67076, 67102, 44181, 67084FL, or 67084alt substrate, as determined *in vivo* or *in vitro*, according to standard techniques. In one embodiment, a phospholipid transporter activity is a direct activity, such as an association with a 67076, 67102, 44181, 67084FL, or 67084alt target molecule. As used herein, a "target molecule" or "binding partner" is a molecule with which a 67076, 67102, 44181, 67084FL, or 67084alt protein binds or interacts in nature, such that 67076, 67102, 44181, 67084FL, or 67084alt-mediated function is achieved. In an exemplary embodiment, a 67076, 67102, 44181, 67084FL, or 67084alt target molecule is a 67076, 67102, 44181, 67084FL, or 67084alt substrate (*e.g.,* a phospholipid, ATP, or a non-67076, 67102, 44181, 67084FL, or 67084alt protein). A phospholipid transporter activity can also be an indirect activity, such as a cellular signaling activity mediated by interaction of the 67076, 67102, 44181, 67084FL, or 67084alt protein with a 67076, 67102, 44181, 67084FL, or 67084alt substrate.

In a preferred embodiment, a phospholipid transporter activity is at least one of the following activities: (i) interaction with a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (e.g., a phospholipid, ATP, or a non-67076, 67102, 44181,

67084FL, or 67084alt protein); (ii) transport of a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (*e.g.*, an aminophospholipid such as phosphatidylserine or phosphatidylethanolamine) from one side of a cellular membrane to the other; (iii) the ability to be phosphorylated or dephosphorylated; (iv) adoption of an E1 conformation or an E2 conformation; (v) conversion of a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule to a product (*e.g.*, hydrolysis of ATP); (vi) interaction with a second non-67076, 67102, 44181, 67084FL, or 67084alt protein; (vii) modulation of substrate or target molecule location (e.g., modulation of phospholipid location within a cell and/or location with respect to a cellular membrane); (viii) maintenance of aminophospholipid gradients; (ix) modulation of intra- or intercellular signaling and/or gene transcription (*e.g.*, either directly or indirectly); and/or (x) modulation of cellular proliferation, growth, differentiation, apoptosis, absorption, or secretion.

The nucleotide sequence of the isolated human 67076, 67102, 44181, 67084FL, or 67084alt cDNA and the predicted amino acid sequence of the human 67076, 67102, 44181, 67084FL, or 67084alt polypeptides are shown in Figures 20A-E, 24A-E, 28A-E, 32A-E, and 36A-E, and in SEQ ID NOs:13 and 14, SEQ ID NOs:16 and 17, SEQ ID NOs:19 and 20, SEQ ID NOs:22 and 23, and SEQ ID NOs:25 and 26, respectively. Plasmids containing the nucleotide sequence encoding human 67076, human 67102, human 44181, human 67084FL, and/or human 67084alt were deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, _____, _____, _____, and _____, respectively, and assigned Accession Numbers _____, _____, _____, _____, and _____, respectively. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposit were made merely as a convenience for those of skill in the art and are not admissions that a deposit is required under 35 U.S.C. §112.

The human 67076 gene, which is approximately 6582 nucleotides in length, encodes a polypeptide which is approximately 1129 amino acid residues in length. The human 67102 gene, which is approximately 6074 nucleotides in length, encodes a polypeptide which is approximately 1426 amino acid residues in length. The human 44181 gene, which is approximately 7221 nucleotides in length, encodes a polypeptide which is approximately 1177 amino acid residues in length. The human 67084FL gene, which is approximately 4198 nucleotides in length, encodes a polypeptide which is approximately 1084 amino acid residues in length. The human 67084alt gene, which is approximately 4231 nucleotides in length, encodes a polypeptide which is approximately 1095 amino acid residues in length.

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt-encoding nucleic acid molecules (*e.g.*, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA) and fragments for use as PCR primers for the amplification or mutation of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ or a portion thereof, can be isolated using standard

molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, as a hybridization probe, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In one embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the human 8099 cDNA. This cDNA comprises sequences encoding the human 8099 polypeptide (*i.e.*, "the coding region", from nucleotides 180-2034) as well as 5' untranslated sequences (nucleotides 1-179) and 3' untranslated sequences (nucleotides 2035-2725). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 180-2034, corresponding to SEQ ID NO:3). Accordingly, in another

embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:3 and nucleotides 1-179 and 2035-2725 of SEQ ID NO:1. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3.

In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:4. The sequence of SEQ ID NO:4 corresponds to the human 46455 cDNA. This cDNA comprises sequences encoding the human 46455 polypeptide (*i.e.*, "the coding region", from nucleotides 376-1963) as well as 5' untranslated sequences (nucleotides 1-375) and 3' untranslated sequences (nucleotides 1964-2230). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:4 (*e.g.*, nucleotides 376-1963, corresponding to SEQ ID NO:6). Accordingly, in another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:6 and nucleotides 1-375 and 1964-2230 of SEQ ID NO:4. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:4 or SEQ ID NO:6.

In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:7. This cDNA may comprise sequences encoding the human 54414 protein (*e.g.*, the "coding region", from nucleotides 225-3578), as well as 5' untranslated sequence (nucleotides 1-224) and 3' untranslated sequences (nucleotides 3579-4632) of SEQ ID NO:7. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:7 (*e.g.*, nucleotides 225-3578, corresponding to SEQ ID NO:9). Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention comprises SEQ ID NO:9 and nucleotides 1-224 of SEQ ID NO:7. In yet another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:9 and nucleotides 3579-4632 of SEQ ID NO:7. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:7 or SEQ ID NO:9.

In still another embodiment, the cDNA may comprise sequences encoding the human 53763 protein (*e.g.*, the "coding region", from nucleotides 561-2474), as well as 5' untranslated sequence (nucleotides 1-560) and 3' untranslated sequences (nucleotides 2475-2847) of SEQ ID NO:10. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:10 (*e.g.*, nucleotides 561-2474, corresponding to SEQ ID NO:6). Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention comprises SEQ ID NO:12 and nucleotides 1-560 of SEQ ID NO:10. In yet another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:12 and nucleotides 2475-2847 of SEQ ID NO:10. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:10 or SEQ ID NO:12.

In yet another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:13. The sequence of SEQ ID

NO:13 corresponds to the human 67076 cDNA. This cDNA comprises sequences encoding the human 67076 polypeptide (*i.e.*, "the coding region", from nucleotides 524-3910) as well as 5' untranslated sequences (nucleotides 1-523) and 3' untranslated sequences (nucleotides 3911-6582). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:13 (*e.g.*, nucleotides 524-3910, corresponding to SEQ ID NO:15). Accordingly, in another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:15 and nucleotides 1-523 or 3911-6582 of SEQ ID NO:13. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:13 or SEQ ID NO:15.

In another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:16. The sequence of SEQ ID NO:16 corresponds to the human 67102 cDNA. This cDNA comprises sequences encoding the human 67102 polypeptide (*i.e.*, "the coding region", from nucleotides 274-4551) as well as 5' untranslated sequences (nucleotides 1-273) and 3' untranslated sequences (nucleotides 4552-6074). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:16 (*e.g.*, nucleotides 274-4551, corresponding to SEQ ID NO:18). Accordingly, in another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:18 and nucleotides 1- 273 or 4552-6074 of SEQ ID NO:16. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:16 or SEQ ID NO:18.

In still another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:19. The sequence of SEQ ID NO:19 corresponds to the human 44181 cDNA. This cDNA comprises sequences encoding the human 44181 polypeptide (*i.e.*, "the coding region", from nucleotides 167-3697) as well as 5' untranslated sequences (nucleotides 1-166) and 3' untranslated sequences (nucleotides 3698-7221). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:19 (*e.g.*, nucleotides 167-3697, corresponding to SEQ ID NO:21). Accordingly, in another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:21 and nucleotides 1-166 or 3698-7221 of SEQ ID NO:19. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:19 or SEQ ID NO:21.

In yet another embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:22. The sequence of SEQ ID NO:22 corresponds to the human 67084FL cDNA. This cDNA comprises sequences encoding the human 67084FL polypeptide (*i.e.*, "the coding region", from nucleotides 156-3407) as well as 5' untranslated sequences (nucleotides 1-155) and 3' untranslated sequences (nucleotides 3408-4198). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:22 (*e.g.*, nucleotides 156-3407, corresponding to SEQ

ID NO:24). Accordingly, in another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:24 and nucleotides 1-155 or 3408-4198 of SEQ ID NO:22. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:22 or SEQ ID NO:24.

In a further embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:25. The sequence of SEQ ID NO:25 corresponds to the human 67084alt cDNA. This cDNA comprises sequences encoding the human 67084alt polypeptide (*i.e.*, "the coding region", from nucleotides 156-3440) as well as 5' untranslated sequences (nucleotides 1-155) and 3' untranslated sequences (nucleotides 3441-4231). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:25 (*e.g.*, nucleotides 156-3440, corresponding to SEQ ID NO:27). Accordingly, in another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:27 and nucleotides 1-155 or 3441-4231 of SEQ ID NO:25. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:25 or SEQ ID NO:27.

In still another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22,

SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27 (*e.g.*, to the entire length of the nucleotide sequence), or to the nucleotide sequence (*e.g.*, the entire length of the nucleotide sequence) of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, or a portion of any of these nucleotide sequences. In one embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least (or no greater than) 50-100, 100-250, 250-500, 500-750, 750-1000, 1000-1250, 1250-1500, 1500-1750, 1750-2000, 2000-2250, 2250-2500, 2500-2750, 2750-3000, 3000-3250, 3250-3500, 3500-3750, 3750-4000, 4000-4250, 4250-4500, 4500-4750, 4750-5000, 5000-5250, 5250-5500, 5500-5750, 5750-6000, 6000-6250, 6250-6500, 6500-6750, 6750-7000, 7000-7250, 7250-7500 or more nucleotides in length and hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ .

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, *e.g.*, a biologically active portion of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. The nucleotide sequence determined from the cloning of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene allows for the generation of probes and primers designed for use in identifying and/or cloning other 8099, 46455, 54414, 53763, 67076,

67102, 44181, 67084FL, or 67084alt family members, as well as 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The probe/primer (*e.g.*, oligonucleotide) typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, 75, 80, 85, 90, 95, or 100 or more consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____.

Exemplary probes or primers are at least 12, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or more nucleotides in length and/or comprise consecutive nucleotides of an isolated nucleic acid molecule described herein. Probes based on the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences can be used to detect (*e.g.*, specifically detect) transcripts or genomic sequences encoding the same or homologous polypeptides. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. In another embodiment a set of primers is provided, *e.g.*, primers suitable for use in a PCR, which can be used to amplify a selected region of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence, *e.g.*, a domain, region, site or other sequence described herein. The primers should be at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides in length. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, such as by measuring a level of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or

67084alt -encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA levels or determining whether a genomic 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, which encodes a polypeptide having a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt biological activity (the biological activities of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides are described herein), expressing the encoded portion of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. In an exemplary embodiment, the nucleic acid molecule is at least 50-100, 100-250, 250-500, 500-750, 750-1000, 1000-1250, 1250-1500, 1500-1750, 1750-2000, 2000-2250, 2250-2500, 2500-2750, 2750-3000, 3000-3250, 3250-3500, 3500-3750, 3750-4000, 4000-4250, 4250-4500, 4500-4750, 4750-5000, 5000-5250, 5250-5500, 5500-5750, 5750-6000, 6000-6250, 6250-6500, 6500-6750, 6750-7000, 7000-7250, 7250-7500 or more nucleotides in length and encodes a polypeptide having a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity (as described herein).

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____,or _____. Such differences can be due to due to degeneracy of the genetic code, thus resulting in a nucleic acid which encodes the same 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides as those encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA

insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ . In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a polypeptide having an amino acid sequence which differs by at least 1, but no greater than 5, 10, 20, 50 or 100 amino acid residues from the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____, _____, _____, _____, or _____. In yet another embodiment, the nucleic acid molecule encodes the amino acid sequence of human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . If an alignment is needed for this comparison, the sequences should be aligned for maximum homology.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologues (different locus), and orthologues (different organism) or can be non naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

Allelic variants result, for example, from DNA sequence polymorphisms within a population (*e.g.*, the human population) that lead to changes in the amino acid sequences of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides. Such genetic polymorphism in the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, preferably a mammalian 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, and can further include non-coding regulatory sequences, and introns.

Accordingly, in one embodiment, the invention features isolated nucleic acid molecules which encode a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ , wherein the nucleic acid molecule hybridizes to a complement of a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18,

SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, for example, under stringent hybridization conditions.

Allelic variants of human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt include both functional and non-functional 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides.

Functional allelic variants are naturally occurring amino acid sequence variants of the human 8099 or 46455 polypeptides that have an 8099 or 46455 activity, *e.g.*, maintain the ability to bind an 8099 or 46455 ligand or substrate and/or modulate sugar transport, or sugar homeostasis.

Functional allelic variants are naturally occurring amino acid sequence variants of the human 54414 or 53763 polypeptides that maintain the ability to, *e.g.*, bind or interact with a 54414 or 53763 target molecule and/or modulate membrane excitability.

Functional allelic variants are naturally occurring amino acid sequence variants of the human 67076, 67102, 44181, 67084FL, or 67084alt polypeptides that have a 67076, 67102, 44181, 67084FL, or 67084alt activity, *e.g.*, bind or interact with a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule, transport a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule across a cellular membrane, hydrolyze ATP, be phosphorylated or dephosphorylated, adopt an E1 conformation or an E2 conformation, and/or modulate cellular signaling, growth, proliferation, differentiation, absorption, or secretion.

Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, or substitution, deletion or insertion of non-critical residues in non-critical regions of the polypeptide.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human 8099 or 46455 polypeptides that do not have a 8099 or 46455 activity, *e.g.*, maintain the ability to bind an 8099 or 46455 ligand or substrate and/or modulate sugar transport, or sugar homeostasis.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human 54414 or 53763 polypeptides that do not maintain the ability to, *e.g.*, bind or interact with a 54414 or 53763 target molecule and/or modulate membrane excitability.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human 67076, 67102, 44181, 67084FL, or 67084alt polypeptides that do not have a 67076, 67102, 44181, 67084FL, or 67084alt activity, *e.g.*, that do not have the ability to, *e.g.*, bind or interact with a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule, transport a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule across a cellular membrane, hydrolyze ATP, be phosphorylated or

dephosphorylated, adopt an E1 conformation or an E2 conformation, and/or modulate cellular signaling, growth, proliferation, differentiation, absorption, or secretion.

Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human orthologues of the human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides. Orthologues of human 8099 or 46455 polypeptides are polypeptides that are isolated from non-human organisms and possess the same 8099 and/or 46455 activity, *e.g.*, ligand binding and/or modulation of sugar transport mechanisms, as the human 8099 and/or 46455 polypeptide. Orthologues of the human 54414 or 53763 polypeptides are polypeptides that are isolated from non-human organisms and possess the same 54414 or 53763 target molecule binding mechanisms and/or ability to modulate membrane excitability of the human 54414 or 53763 polypeptides. Orthologues of human 67076, 67102, 44181, 67084FL, or 67084alt polypeptides are polypeptides that are isolated from non-human organisms and possess the same 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule binding mechanisms, phospholipid transporting activity, ATPase activity, and/or modulation of cellular signaling mechanisms of the human 67076, 67102, 44181, 67084FL, or 67084alt proteins as the human 67076, 67102, 44181, 67084FL, or 67084alt polypeptides.

Orthologues of the human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26.

Moreover, nucleic acid molecules encoding other 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt family members and, thus, which have a nucleotide sequence which differs from the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ are intended to be within the scope of the invention. For example, another 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt cDNA can be identified based on the nucleotide sequence of human

8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . Moreover, nucleic acid molecules encoding 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides from different species, and which, thus, have a nucleotide sequence which differs from the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ are intended to be within the scope of the invention. For example, a mouse 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt cDNA can be identified based on the nucleotide sequence of a human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt .

Nucleic acid molecules corresponding to natural allelic variants and homologues of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt cDNAs of the invention can be isolated based on their homology to the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural allelic variants and homologues of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene.

Orthologues, homologues and allelic variants can be identified using methods known in the art (*e.g.*, by hybridization to an isolated nucleic acid molecule of the present invention, for example, under stringent hybridization conditions). In one embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____. In other embodiment, the nucleic acid is at least 50-100, 100-250, 250-500, 500-750, 750-1000, 1000-1250, 1250-1500, 1500-1750, 1750-2000, 2000-2250, 2250-2500, 2500-2750, 2750-3000, 3000-3250, 3250-3500, 3500-3750, 3750-4000, 4000-4250, 4250-4500, 4500-4750, 4750-5000, 5000-5250, 5250-5500, 5500-5750,

5750-6000, 6000-6250, 6250-6500, 6500-6750, 6750-7000, 7000-7250, 7250-7500 or more nucleotides in length.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C) = 81.5 + 16.6($\log_{10}[Na^+]$) + 0.41(%G+C) - (600/N), where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional

preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65°C, see *e.g.*, Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, and corresponds to a naturally-occurring nucleic acid

molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural polypeptide).

In addition to naturally-occurring allelic variants of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____, thereby leading to changes in the amino acid sequence of the encoded 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, without altering the functional ability of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt (*e.g.*, the sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity.

For example, amino acid residues that are conserved among the 8099 or 46455 polypeptides of the present invention, *e.g.*, those present in a transmembrane domain and/or a

sugar transporter family domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the 8099 or 46455 polypeptides of the present invention and other members of the 8099 or 46455 family are not likely to be amenable to alteration.

Amino acid residues that are conserved among the 54414 or 53763 polypeptides of the present invention, *e.g.*, those present in a P-loop or a pore domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the 54414 or 53763 polypeptides of the present invention and other members of the potassium channel family are not likely to be amenable to alteration.

Amino acid residues that are conserved among the 67076, 67102, 44181, 67084FL, or 67084alt polypeptides of the present invention, *e.g.*, those present in a E1-E2 ATPases phosphorylation site, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the 67076, 67102, 44181, 67084FL, or 67084alt polypeptides of the present invention and other members of the phospholipid transporter family are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides that contain changes in amino acid residues that are not essential for activity. Such 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides differ in amino acid sequence from SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26 (*e.g.*, to the entire length of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26).

An isolated nucleic acid molecule encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide identical to the polypeptide of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession

Number ______, _______, _______, _______, or _______, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded polypeptide. Mutations can be introduced into SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number ______, _______, _______, _______, or _______ by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number ______, ______, _____, _____, _____, _______, _______, _______, or _______, the encoded polypeptide can be expressed recombinantly and the activity of the polypeptide can be determined.

In a preferred embodiment, a mutant 8099 and/or 46455 polypeptide can be assayed for the ability to (1) bind a monosaccharide, *e.g.*, D-glucose, D-fructose, D-galactose, and/or mannose, (2) transport monosaccharides across a cell membrane, (3) influence insulin and/or glucagon secretion, (4) maintain sugar homeostasis in a cell, (5) function as a neuronal transporter, and (6) mediate trans-epithelial movement in a cell.

In another preferred embodiment, a mutant 54414 and/or 53763 protein can be assayed for the ability to (i) interact with a 54414 and/or 53763 substrate (*e.g.*, a potassium ion or a cyclic nucleotide); (ii) conduct or transport a 54414 and/or 53763 substrate across a cellular membrane; (iii) interact with a second non-54414 and/or 53763 protein (*e.g.*, a 54414 and/or 53763 polypeptide or a 54414 and/or 53763 -potassium channel subunit); (iv) modulate (*e.g.*, maintain and/or rectify) membrane potentials; (v) regulate target molecule availability or activity; (vi) modulate intra- or intercellular signaling and/or gene transcription (*e.g.*, either directly or indirectly); (viii) generate outwardly rectifying currents; (viii) modulate membrane excitability; (ix) modulate the release of neurotransmitters; (x) regulate contractility *(e.g.,* of smooth muscle cells), secretion, and/or synaptic transmission; and/or (xi) modulate processes which underlie learning and memory.

In a further preferred embodiment, a mutant 54414 protein can be assayed for the ability to (i) interact with maxi-K potassium channels (i.e., large conductance channels, in particular *Slo*); (ii) modulate maxi-K potassium channel activity (e.g., *Slo*-mediated activities); (iii) generate intermediate conductance channels; and/or (iv) regulate contractility (e.g., of smooth muscle cells), secretion, and/or synaptic transmission, in particular, via modulation of *Slo*.

In still a further preferred embodiment, a mutant 53763 protein can be assayed for the ability to (i) interact with Shaker (Sh) potassium channels and/or channel subunits; (ii) modulate Shaker (Sh) potassium channel activity (e.g., termination of prolonged membrane depolarization); and/or (iii) modulation of high voltage activating channel activity and/or inactivating channel activity, and the like.

In yet another preferred embodiment, a mutant 67076, 67102, 44181, 67084FL, and/or 67084alt polypeptide can be assayed for the ability to (i) interact with a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (*e.g.,* a phospholipid, ATP, or a non-67076, 67102, 44181, 67084FL, or 67084alt protein); (ii) transport a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (*e.g.*, an aminophospholipid such as phosphatidylserine or phosphatidylethanolamine) from one side of a cellular membrane to the other; (iii) be phosphorylated or dephosphorylated; (iv) adopt an E1 conformation or an E2 conformation; (v) convert a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule to a product (*e.g.*, hydrolysis of ATP); (vi) interact with a second non-67076, 67102, 44181, 67084FL, or 67084alt protein; (vii) modulate substrate or target molecule location (*e.g.*, modulation of phospholipid location within a cell and/or location with respect to a cellular membrane); (viii) maintain aminophospholipid gradients; (ix) modulate intra- or intercellular signaling and/or gene transcription (*e.g.*, either directly or indirectly); and/or (x) modulate cellular proliferation, growth, differentiation, apoptosis, absorption, or secretion.

In addition to the nucleic acid molecules encoding 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. In an exemplary embodiment, the invention provides an isolated nucleic acid molecule which is antisense to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule (*e.g.*, is antisense to the coding strand of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule). An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a polypeptide, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt corresponds to SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, and SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, and SEQ ID NO:27, respectively). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt disclosed herein (*e.g.*, SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, and SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, and SEQ ID NO:27), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA (*e.g.*, between the –10 and +10 regions of the start site of a gene nucleotide sequence). An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50

nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide to thereby inhibit expression of the polypeptide, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve

sufficient intra-cellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA transcripts to thereby inhibit translation of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA. A ribozyme having specificity for a 8099-, 46455-, 54414-, 53763-, 67076-, 67102-, 44181-, 67084FL-, or 67084alt -encoding nucleic acid can be designed based upon the nucleotide sequence of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt cDNA disclosed herein (*i.e.*, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 8099-, 46455-, 54414-, 53763-, 67076-, 67102-, 44181-, 67084FL-, or 67084alt -encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt (*e.g.*, the 8099, 46455, 54414, 53763, 67076, 67102, 44181,

67084FL, or 67084alt promoter and/or enhancers) to form triple helical structures that prevent transcription of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al. Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of

bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl) amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

Alternatively, the expression characteristics of an endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene. For example, an endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene which is normally "transcriptionally silent", *i.e.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene which is normally not expressed, or is expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, using techniques, such as

targeted homologous recombination, which are well known to those of skill in the art, and described, *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

II. Isolated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt Polypeptides and Anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt Antibodies

One aspect of the invention pertains to isolated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt or recombinant polypeptides and polypeptides, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibodies. In one embodiment, native 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides are produced by recombinant DNA techniques. Alternative to recombinant expression, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide in which the polypeptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide having less than about 30% (by dry weight) of non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, still more preferably less than about 10% of non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, and most preferably less than about 5% non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. When the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of

culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide in which the polypeptide is separated from chemical precursors or other chemicals which are involved in the synthesis of the polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide having less than about 30% (by dry weight) of chemical precursors or non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt chemicals, more preferably less than about 20% chemical precursors or non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt chemicals, still more preferably less than about 10% chemical precursors or non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt chemicals, and most preferably less than about 5% chemical precursors or non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt chemicals.

As used herein, a "biologically active portion" of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide includes a fragment of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide which participates in an interaction between a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecule and a non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecule (*e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate). Biologically active portions of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, which include less amino acids than the full length 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, and exhibit at least one activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide.

Typically, biologically active portions of a 8099 or 46455 polypeptide comprise a domain or motif with at least one activity of the 8099 or 46455 polypeptide, *e.g.*, modulating sugar transport mechanisms. A biologically active portion of an 8099 polypeptide can be a polypeptide which is, for example, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 600 or more amino acids in

length. A biologically active portion of an 46455 polypeptide can be a polypeptide which is, for example, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525 or more amino acids in length. Biologically active portions of an 8099 and/or an 46455 polypeptide can be used as targets for developing agents which modulate an 8099 or 46455 mediated activity, *e.g.*, a sugar transport mechanism.

In one embodiment, a biologically active portion of an 8099 or an 46455 polypeptide comprises at least one transmembrane domain. It is to be understood that a preferred biologically active portion of an 8099 or an 46455 polypeptide of the present invention comprises at least one or more of the following domains: a transmembrane domain and/or a sugar transporter family domain. Moreover, other biologically active portions, in which other regions of the polypeptide are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 8099 or 46455 polypeptide.

Moreover, biologically active portions of a 54414 or 53763 polypeptide comprise a domain or motif with at least one activity of the 54414 or 53763 polypeptide, *e.g.*, modulation of intra- or inter-cellular signaling and/or gene expression, and/or modulate membrane excitability. A biologically active portion of a 54414 or 53763 polypeptide can be a polypeptide which is, for example, 10, 25, 50, 75, 100, 125, 150 or more amino acids in length. Biologically active portions of a 54414 or 53763 polypeptide can be used as targets for developing agents which modulate a 54414 or 53763 mediated activity, *e.g.*, modulation of intra- or inter-cellular signaling and/or gene expression, and/or modulate membrane excitability.

In one embodiment, a biologically active portion of a 54414 or 53763 polypeptide comprises at least one transmembrane domain and/or a pore domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 54414 or 53763 polypeptide.

Biologically active portions of a 67076, 67102, 44181, 67084FL, or 67084alt polypeptide comprise a domain or motif with at least one activity of the 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, *e.g.*, the ability to interact with a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (*e.g.*, a phospholipid; ATP; a non-67076, 67102, 44181, 67084FL, or 67084alt protein; or another 67076, 67102, 44181, 67084FL, or 67084alt protein or subunit); the ability to transport a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (*e.g.*, a phospholipid) from one side of a cellular membrane to the other; the ability to be phosphorylated or dephosphorylated; the ability to adopt an E1 conformation or an E2 conformation; the ability to convert a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule to a product (*e.g.*, the ability to hydrolyze ATP); the ability to interact with a second non-67076, 67102, 44181,

67084FL, or 67084alt protein; the ability to modulate intra- or inter-cellular signaling and/or gene transcription (*e.g.*, either directly or indirectly); the ability to modulate cellular growth, proliferation, differentiation, absorption, and/or secretion. A biologically active portion of a 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be a polypeptide which is, for example, 10, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550 or more amino acids in length. Biologically active portions of a 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be used as targets for developing agents which modulate a 67076, 67102, 44181, 67084FL, or 67084alt mediated activity, *e.g.*, modulating transport of biological molecules across membranes.

In one embodiment, a biologically active portion of a 67076, 67102, 44181, 67084FL, or 67084alt polypeptide comprises at least one at least one or more of the following domains, sites, or motifs: a transmembrane domain, an N-terminal large extramembrane domain, a C-terminal large extramembrane domain, an E1-E2 ATPases phosphorylation site, a P-type ATPase sequence 1 motif, a P-type ATPase sequence 2 motif, a P-type ATPase sequence 3 motif, and/or one or more phospholipid transporter specific amino acid resides. Moreover, other biologically active portions, in which other regions of the polypeptide are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 67076, 67102, 44181, 67084FL, or 67084alt polypeptide.

Another aspect of the invention features fragments of the polypeptide having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, for example, for use as immunogens. In one embodiment, a fragment comprises at least 5 amino acids (*e.g.*, contiguous or consecutive amino acids) of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____, _____, _____, _____, or _____. In another embodiment, a fragment comprises at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or more amino acids (*e.g.*, contiguous or consecutive amino acids) of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____, _____, _____, _____, or _____.

In a preferred embodiment, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide has an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID

NO:20, SEQ ID NO:23, or SEQ ID NO:26. In other embodiments, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide is substantially identical to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, and retains the functional activity of the polypeptide of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. In another embodiment, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide is a polypeptide which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26.

In another embodiment, the invention features a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or a complement thereof. This invention further features a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or a complement thereof.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the 8099 amino acid sequence of SEQ ID NO:2 having 617 amino acid residues, at least 185,

preferably at least 246, more preferably at least 308, more preferably at least 370, even more preferably at least 431, and even more preferably at least 493 or 555 or more amino acid residues are aligned. In another preferred embodiment, the sequences being aligned for comparison purposes are globally aligned and percent identity is determined over the entire length of the sequences aligned. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at the Accelrys website), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in conjunction with the GAP program include a Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or version 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and polypeptide sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules of the

invention. BLAST protein searches can be performed with the XBLAST program, score = 100, wordlength = 3, and a Blosum62 matrix to obtain amino acid sequences homologous to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See the National Center for Biotechnology website.

The invention also provides 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt chimeric or fusion proteins. As used herein, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt "chimeric protein" or "fusion protein" comprises a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide operatively linked to a non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. A "8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide whereas a "non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not substantially homologous to the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, respectively, *e.g.*, a polypeptide which is different from the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide and which is derived from the same or a different organism. Within a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fusion protein the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can correspond to all or a portion of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. In a preferred embodiment, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fusion protein comprises at least one biologically active portion of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. In another preferred embodiment, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fusion protein comprises at least two biologically active portions of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. Within the fusion protein, the term "operatively linked" is intended to indicate that the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide and the non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide are fused in-frame to each other. The non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be fused to the N-terminus or C-terminus of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide.

For example, in one embodiment, the fusion protein is a GST-8099, -46455, -54414, -53763, -67076, -67102, -44181, -67084FL, or -67084alt fusion protein in which the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt .

In another embodiment, the fusion protein is a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be increased through the use of a heterologous signal sequence.

The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fusion proteins can be used to affect the bioavailability of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate. Use of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide; (ii) mis-regulation of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene; and (iii) aberrant post-translational modification of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide.

Moreover, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -fusion proteins of the invention can be used as immunogens to produce anti-8099, anti-46455, anti-54414, anti-53763, anti-67076, anti-67102, anti-44181, anti-67084FL, and/or anti-67084alt antibodies in a subject, to purify 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt ligands and in screening assays to identify molecules which inhibit the interaction with or transport of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate.

Preferably, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques

including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide.

The present invention also pertains to variants of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides which function as either 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt agonists (mimetics) or as 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antagonists. Variants of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. An agonist of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. An antagonist of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can inhibit one or more of the activities of the naturally occurring form of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide by, for example, competitively modulating a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -mediated activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the polypeptide has fewer side effects in a subject relative to treatment with the naturally occurring form of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide.

In one embodiment, variants of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide which function as either 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt agonists (mimetics) or as 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide for 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide agonist or

antagonist activity. In one embodiment, a variegated library of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences therein. There are a variety of methods which can be used to produce libraries of potential 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide coding sequence can be used to generate a variegated population of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fragments for screening and subsequent selection of variants of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of 8099, 46455, 54414,

53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA 89*:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt library. For example, a library of expression vectors can be transfected into a cell line, which ordinarily responds to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt in a particular 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate-dependent manner. The transfected cells are then contacted with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt and the effect of the expression of the mutant on signaling by the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate can be detected, *e.g.*, phospholipid transport (*e.g.*, by measuring phospholipid levels inside the cell or its various cellular compartments, within various cellular membranes, or in the extra-cellular medium), hydrolysis of ATP, phosphorylation or dephosphorylation of the HEAT protein, and/or gene transcription. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the HEAT substrate, or which score for increased or decreased levels of phospholipid transport or ATP hydrolysis, and the individual clones further characterized.

An isolated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt using standard techniques for polyclonal and monoclonal antibody preparation. A full-length 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be used or, alternatively, the invention provides antigenic peptide fragments of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt for use as immunogens. The antigenic peptide of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, or SEQ ID NO:26 and encompasses an epitope of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt such

that an antibody raised against the peptide forms a specific immune complex with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt that are located on the surface of the polypeptide, *e.g.*, hydrophilic regions, as well as regions with high antigenicity (see, for example, Figures 2, 9, 13, 17, 21, 25, 29, 33, and 37).

A 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or a chemically synthesized 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt preparation induces a polyclonal anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody response.

Accordingly, another aspect of the invention pertains to polyclonal anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . A monoclonal antibody composition thus typically displays a single binding affinity for a particular 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide with which it immunoreacts.

Polyclonal anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibodies can be prepared as described above by immunizing a suitable subject

with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt immunogen. The anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . If desired, the antibody molecules directed against 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem* .255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.,* 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt .

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al. Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture

medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt , *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt to thereby isolate immunoglobulin library members that bind 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al. Nature* (1990) 348:552-554.

Additionally, recombinant anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and

humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody (*e.g.*, monoclonal antibody) can be used to isolate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody can facilitate the purification of natural 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt from cells and of recombinantly produced 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expressed in host cells. Moreover, an anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody can be used to detect 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. Anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibodies can be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include

luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, for example recombinant expression vectors, containing a nucleic acid containing a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule or vectors containing a nucleic acid molecule which encodes a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide

sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, mutant forms of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, fusion proteins, and the like).

Accordingly, an exemplary embodiment provides a method for producing a polypeptide, preferably a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, by culturing in a suitable medium a host cell of the invention (*e.g.*, a mammalian host cell such as a non-human mammalian cell) containing a recombinant expression vector, such that the polypeptide is produced.

The recombinant expression vectors of the invention can be designed for expression of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides in prokaryotic or eukaryotic cells. For example, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5

(Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, for example. In a preferred embodiment, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9

cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are

produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule of the invention is introduced, *e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule within a vector (*e.g.*, a recombinant expression vector) or a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or

67084alt polypeptide or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide. Accordingly, the invention further provides methods for producing a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide has been introduced) in a suitable medium such that a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide is produced. In another embodiment, the method further comprises isolating a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences have been introduced into their genome or homologous recombinant animals in which endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences have been altered. Such animals are useful for studying the function and/or activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt and for identifying and/or evaluating modulators of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt cDNA sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, or SEQ ID NO:13 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, such as a mouse or rat 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, can be used as a transgene. Alternatively, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene homologue, such as another 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt family member, can be isolated based on hybridization to the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt cDNA sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt transgene to direct expression of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt transgene in its genome and/or expression of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene. The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, or SEQ ID NO:15), but more preferably, is a non-human homologue of a human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene (*e.g.*, a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, or SEQ ID NO:13). For example, a mouse 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene is mutated or otherwise altered but still encodes functional polypeptide (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide). In the homologous recombination nucleic acid molecule, the altered portion of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene to allow for homologous recombination to occur between the exogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene carried by the homologous recombination nucleic acid molecule and an endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in

which the introduced 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene has homologously recombined with the endogenous 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

IV. Pharmaceutical Compositions

The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules, fragments of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibodies, and or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt modulators, (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, polypeptide, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol,

propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or an anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such

compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a polypeptide or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*,. including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or

inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly

actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, antibodies, and modulators described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic).

As described herein, an 8099 and/or 46455 polypeptide of the invention has one or more of the following activities: (1) bind a monosaccharide, *e.g.*, D-glucose, D-fructose, D-galactose, and/or mannose, (2) transport monosaccharides across a cell membrane, (3) influence insulin and/or glucagon secretion, (4) maintain sugar homeostasis in a cell, (5) function as a neuronal transporter, and (6) mediate trans-epithelial movement in a cell.

As described herein, a 54414 and/or 53763 protein of the invention has one or more of the following activities: (i) interaction with a 54414 or 53763 substrate (*e.g.*, a potassium ion or a cyclic nucleotide); (ii) conductance or transport of a 54414 or 53763 substrate across a cellular membrane; (iii) interaction with a second non-54414 or 53763 protein (e.g., a 54414 or 53763 polypeptide or a non-54414 or 53763 potassium channel subunit); (iv) modulation (e.g., maintenance and/or rectification) of membrane potentials; (v) regulation of target molecule availability or activity; (vi) modulation of intra- or intercellular signaling and/or gene transcription (*e.g.*, either directly or indirectly); (viii) generation of outwardly rectifying currents; (viii) modulation of membrane excitability; (ix) modulation of the release of neurotransmitters; (x) regulation of contractility (e.g., of smooth muscle cells), secretion, and/or synaptic transmission; and/or (xi) modulation of processes which underlie learning and memory.

Preferred activities of 54414 further include at least one of the following activities: (i) interaction with maxi-K potassium channels (i.e., large conductance channels, in particular *Slo*); (ii) modulation of maxi-K potassium channel activity (e.g., *Slo*-mediated activities); (iii) generation of intermediate conductance channels; and/or (iv) regulation of contractility (e.g., of smooth muscle cells), secretion, and/or synaptic transmission, in particular, via modulation of *Slo*.

Preferred activities of 53763 further include at least one of the following activities: (i) interaction with Shaker (Sh) potassium channels and/or channel subunits; (ii) modulation of Shaker (Sh) potassium channel activity (e.g., termination of prolonged membrane depolarization; (iii) modulation of high voltage activating channel activity and/or inactivating channel activity, and the like.

As described herein, a 67076, 67102, 44181, 67084FL, or 67084alt polypeptide of the invention has one or more of the following activities: (i) interaction with a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule *(e.g.,* a phospholipid, ATP, or a non-67076, 67102, 44181, 67084FL, or 67084alt protein); (ii) transport of a

67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (*e.g.*, an aminophospholipid such as phosphatidylserine or phosphatidylethanolamine) from one side of a cellular membrane to the other; (iii) the ability to be phosphorylated or dephosphorylated; (iv) adoption of an E1 conformation or an E2 conformation; (v) conversion of a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule to a product (*e.g.*, hydrolysis of ATP); (vi) interaction with a second non- 67076, 67102, 44181, 67084FL, or 67084alt protein; (vii) modulation of substrate or target molecule location (*e.g.*, modulation of phospholipid location within a cell and/or location with respect to a cellular membrane); (viii) maintenance of aminophospholipid gradients; (ix) modulation of intra- or intercellular signaling and/or gene transcription (*e.g.*, either directly or indirectly); and/or (x) modulation of cellular proliferation, growth, differentiation, apoptosis, absorption, or secretion.

The isolated nucleic acid molecules of the invention can be used, for example, to express 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA (*e.g.*, in a biological sample) or a genetic alteration in a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, and to modulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity, as described further below. The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can be used to treat disorders characterized by insufficient or excessive production of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate or production or transport of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt inhibitors, for example, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt associated disorders.

As used herein, a "sugar transporter" includes a protein or polypeptide which is involved in transporting a molecule, *e.g.*, a monosaccharide such as D-glucose, D-fructose, D-galactose or mannose, across the plasma membrane of a cell, *e.g.*, a liver cell, fat cell, muscle cell, or blood cell, such as an erythrocyte. Sugar transporters regulate sugar homeostasis in a cell and, typically, have sugar substrate specificity. Examples of sugar transporters include glucose transporters, fructose transporters, and galactose transporters.

As used herein, a "sugar transporter mediated activity" includes an activity which involves a sugar transporter, *e.g.*, a sugar transporter in a liver cell, fat cell, muscle cell, or blood cell, such as an erythrocyte. Sugar transporter mediated activities include the transport of sugars, *e.g.*, D-glucose, D-fructose, D-galactose or mannose, into and out of cells; the stimulation of molecules that regulate glucose homeostasis (*e.g.*, insulin and glucagon), from cells, *e.g.*, pancreatic cells; and the participation in signal transduction pathways associated with sugar metabolism.

As the 8099 and 46455 molecules of the present invention are sugar transporters, they may be useful for developing novel diagnostic and therapeutic agents for sugar transporter associated disorders. As used herein, the terms "sugar transporter associated disorder" and "8099 and 46455 disorder," used interchangeably herein, includes a disorder, disease, or condition which is characterized by an aberrant, *e.g.*, upregulated or downregulated, sugar transporter mediated activity. Sugar transporter associated disorders typically result in, *e.g.*, upregulated or downregulated, sugar levels in a cell. Examples of sugar transporter associated disorders include disorders associated with sugar homeostasis, such as obesity, anorexia, type-1 diabetes, type-2 diabetes, hypoglycemia, glycogen storage disease (Von Gierke disease), type I glycogenosis, bipolar disorder, seasonal affective disorder, and cluster B personality disorders.

As used interchangeably herein, a "potassium channel associated disorder" or a "54414 or 53763 associated disorder" include a disorder, disease or condition which is caused or characterized by a misregulation (*e.g.*, downregulation or upregulation) of 54414 or 53763 activity. 54414 or 53763 associated disorders can detrimentally affect cellular functions such as cellular proliferation, growth, differentiation, inter- or intra-cellular communication; tissue function, such as cardiac function or musculoskeletal function; systemic responses in an organism, such as nervous system responses, hormonal responses (*e.g.*, insulin response), or immune responses; and protection of cells from toxic compounds (*e.g.*, carcinogens, toxins, or mutagens).

In a preferred embodiment, 54414 or 53763 associated disorders include CNS disorders such as cognitive and neurodegenerative disorders, examples of which include, but are not limited to, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, senile dementia, Huntington's disease, Gilles de la Tourette's syndrome, multiple sclerosis, amyotrophic lateral sclerosis, progressive *supra*nuclear palsy, epilepsy, seizure disorders, and Jakob-Creutzfieldt disease; autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders, such as depression, schizophrenia, schizoaffective disorder, korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss, attention deficit disorder, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, phobias, panic disorder, as well as bipolar affective disorder, *e.g.*, severe bipolar affective (mood) disorder (BP-1), and bipolar affective neurological disorders, *e.g.*, migraine and obesity. Further CNS-related disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

54414 or 53763 associated disorders also include cellular proliferation, growth, differentiation, or apoptosis disorders. Cellular proliferation, growth, differentiation, or apoptosis disorders include those disorders that affect cell proliferation, growth, differentiation, or apoptosis processes. As used herein, a "cellular proliferation, growth, differentiation, or apoptosis process" is a process by which a cell increases in number, size or content, by which a cell develops a specialized set of characteristics which differ from that of other cells, or by which a cell undergoes programmed cell death. The 54414 or 53763 molecules of the present invention may modulate cellular growth, proliferation, differentiation, or apoptosis, and may play a role in disorders characterized by aberrantly regulated growth, proliferation, differentiation, or apoptosis. Such disorders include cancer, *e.g.*, carcinoma, sarcoma, or leukemia; tumor angiogenesis and metastasis; skeletal dysplasia; hepatic disorders; and hematopoietic and/or myeloproliferative disorders.

Further examples of 54414 or 53763 associated disorders include cardiac-related disorders. Cardiovascular system disorders in which the 54414 or 53763 molecules of the invention may be directly or indirectly involved include arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrilation, Jervell syndrome, Lange-Nielsen syndrome, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, and arrhythmia. 54414 or 53763 associated disorders also include disorders of the musculoskeletal system such as paralysis and muscle weakness, *e.g.*, ataxia, myotonia, and myokymia.

54414 or 53763 associated or related disorders also include hormonal disorders, such as conditions or diseases in which the production and/or regulation of hormones in an organism is aberrant. Examples of such disorders and diseases include type I and type II diabetes mellitus, pituitary disorders (*e.g.*, growth disorders), thyroid disorders (*e.g.*, hypothyroidism or hyperthyroidism), and reproductive or fertility disorders (*e.g.*, disorders which affect the organs of the reproductive system, *e.g.*, the prostate gland, the uterus, or the vagina; disorders which involve an imbalance in the levels of a reproductive hormone in a subject; disorders affecting the ability of a subject to reproduce; and disorders affecting secondary sex characteristic development, *e.g.*, adrenal hyperplasia).

54414 or 53763 associated or related disorders also include immune disorders, such as autoimmune disorders or immune deficiency disorders, *e.g.*, congenital X-linked infantile hypogammaglobulinemia, transient hypogammaglobulinemia, common variable immunodeficiency, selective IgA deficiency, chronic mucocutaneous candidiasis, or severe combined immunodeficiency.

As used interchangeably herein, a "phospholipid transporter associated disorder" or a "67076, 67102, 44181, 67084FL, or 67084alt associated disorder" includes a disorder, disease or condition which is caused or characterized by a misregulation (*e.g.*, downregulation or upregulation) of 67076, 67102, 44181, 67084FL, or 67084alt activity. 67076, 67102, 44181, 67084FL, or 67084alt associated disorders can detrimentally affect cellular functions such as cellular proliferation, growth, differentiation, inter- or intra-cellular communication; tissue function, such as cardiac function or musculoskeletal function; systemic responses in an organism, such as nervous system responses, hormonal responses (*e.g.*, insulin response), or immune responses; and protection of cells from toxic compounds (*e.g.*, carcinogens, toxins, or mutagens). Examples of 67076, 67102, 44181, 67084FL, or 67084alt associated disorders include CNS disorders such as cognitive and neurodegenerative disorders, examples of which include, but are not limited to, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, senile dementia, Huntington's disease, Gilles de la Tourette's syndrome, multiple sclerosis, amyotrophic lateral sclerosis, progressive *supra*nuclear palsy, epilepsy, seizure disorders, and Jakob-Creutzfieldt disease; autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders, such as depression, schizophrenia, schizoaffective disorder, korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss, attention deficit disorder, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, phobias, panic disorder, as well as bipolar affective disorder, *e.g.*, severe bipolar affective (mood) disorder (BP-1), and bipolar affective neurological disorders, *e.g.*, migraine and obesity. Further CNS-related disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

Further examples of 67076, 67102, 44181, 67084FL, or 67084alt associated disorders include cardiac-related disorders. Cardiovascular system disorders in which the 67076, 67102, 44181, 67084FL, or 67084alt molecules of the invention may be directly or indirectly involved include arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrilation, Jervell syndrome, Lange-Nielsen syndrome, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, and arrhythmia. 67076, 67102, 44181, 67084FL, or 67084alt associated disorders

also include disorders of the musculoskeletal system such as paralysis and muscle weakness, *e.g.*, ataxia, myotonia, and myokymia.

67076, 67102, 44181, 67084FL, or 67084alt associated disorders also include cellular proliferation, growth, or differentiation disorders. Cellular proliferation, growth, or differentiation disorders include those disorders that affect cell proliferation, growth, or differentiation processes. As used herein, a "cellular proliferation, growth, or differentiation process" is a process by which a cell increases in number, size or content, or by which a cell develops a specialized set of characteristics which differ from that of other cells. The 67076, 67102, 44181, 67084FL, or 67084alt molecules of the present invention are involved in phospholipid transport mechanisms, which are known to be involved in cellular growth, proliferation, and differentiation processes. Thus, the 67076, 67102, 44181, 67084FL, or 67084alt molecules may modulate cellular growth, proliferation, or differentiation, and may play a role in disorders characterized by aberrantly regulated growth, proliferation, or differentiation. Such disorders include cancer, *e.g.*, carcinoma, sarcoma, or leukemia; tumor angiogenesis and metastasis; skeletal dysplasia; hepatic disorders; and hematopoietic and/or myeloproliferative disorders.

67076, 67102, 44181, 67084FL, or 67084alt associated or related disorders also include hormonal disorders, such as conditions or diseases in which the production and/or regulation of hormones in an organism is aberrant. Examples of such disorders and diseases include type I and type II diabetes mellitus, pituitary disorders (*e.g.*, growth disorders), thyroid disorders (*e.g.*, hypothyroidism or hyperthyroidism), and reproductive or fertility disorders (*e.g.*, disorders which affect the organs of the reproductive system, *e.g.*, the prostate gland, the uterus, or the vagina; disorders which involve an imbalance in the levels of a reproductive hormone in a subject; disorders affecting the ability of a subject to reproduce; and disorders affecting secondary sex characteristic development, *e.g.*, adrenal hyperplasia).

67076, 67102, 44181, 67084FL, or 67084alt associated or related disorders also include immune disorders, such as autoimmune disorders or immune deficiency disorders, *e.g.*, congenital X-linked infantile hypogammaglobulinemia, transient hypogammaglobulinemia, common variable immunodeficiency, selective IgA deficiency, chronic mucocutaneous candidiasis, or severe combined immunodeficiency.

8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt associated or related disorders also include disorders affecting tissues in which 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt protein is expressed.

In addition, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can be used to screen for naturally occurring 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrates, to screen for drugs or compounds which modulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or

67084alt activity, as well as to treat disorders characterized by insufficient or excessive production of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or production of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide forms which have decreased, aberrant or unwanted activity compared to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt wild type polypeptide (*e.g.*, sugar transporter associated disorder, potassium channel associated diosrders, a phospholipid transporter-associated disorders). Moreover, the anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibodies of the invention can be used to detect and isolate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, to regulate the bioavailability of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, and modulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity.

A. Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides, have a stimulatory or inhibitory effect on, for example, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity is determined.

Determining the ability of the test compound to modulate 8099 or 46455 activity can be accomplished by monitoring, for example, intracellular or extracellular D-glucose, D-fructose, D-galactose, and/or mannose concentration, or insulin or glucagon secretion. The cell, for example, can be of mammalian origin, *e.g.*, a liver cell, fat cell, muscle cell, or a blood cell, such as an erythrocyte.

Determining the ability of the test compound to modulate 54414 or 53763 activity can be accomplished by monitoring, for example, potassium current, neurotransmitter release, and/or membrane excitability in a cell which expresses 54414 or 53763. The cell, for example, can be of mammalian origin, *e.g.*, a neuronal cell.

Determining the ability of the test compound to modulate 67076, 67102, 44181, 67084FL, or 67084alt activity can be accomplished by monitoring, for example, (i) interaction of 67076, 67102, 44181, 67084FL, or 67084alt with a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (*e.g.*, a phospholipid, ATP, or a non-67076, 67102, 44181, 67084FL, or 67084alt protein); (ii) transport of a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule (*e.g.*, an aminophospholipid such as phosphatidylserine or phosphatidylethanolamine) from one side of a cellular membrane to the other; (iii) the ability of 67076, 67102, 44181, 67084FL, or 67084alt to be phosphorylated or dephosphorylated; (iv) adoption by 67076, 67102, 44181, 67084FL, or 67084alt of an E1 conformation or an E2 conformation; (v) conversion of a 67076, 67102, 44181, 67084FL, or 67084alt substrate or target molecule to a product (*e.g.*, hydrolysis of

ATP); (vi) interaction of 67076, 67102, 44181, 67084FL, or 67084alt with a second non-67076, 67102, 44181, 67084FL, or 67084alt protein; (vii) modulation of substrate or target molecule location (e.g., modulation of phospholipid location within a cell and/or location with respect to a cellular membrane); (viii) maintenance of aminophospholipid gradients; (ix) modulation of intra- or intercellular signaling and/or gene transcription (*e.g.*, either directly or indirectly); and/or (x) modulation of cellular proliferation, growth, differentiation, apoptosis, absorption, and/or secretion.

The ability of the test compound to modulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt binding to a substrate or to bind to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can also be determined. Determining the ability of the test compound to modulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt binding to a substrate can be accomplished, for example, by coupling the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate with a radioisotope or enzymatic label such that binding of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be determined by detecting the labeled 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate in a complex. Alternatively, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt binding to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate in a complex. Determining the ability of the test compound to bind 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be accomplished, for example, by coupling the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate with a radioisotope or enzymatic label such that binding of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be determined by detecting the labeled 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate in a complex. Alternatively, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt binding to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate in a complex. Determining the ability of the test compound to bind 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be determined by detecting the labeled 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or

67084alt compound in a complex. For example, compounds (*e.g.*, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate) to interact with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt without the labeling of either the compound or the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt .

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule (*e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate) with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule. Determining the ability of the test compound to modulate the activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule can be accomplished, for example, by determining the cellular location of the target molecule, or by determining whether the target molecule (*e.g.*, ATP) has been hydrolyzed.

Determining the ability of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, or a biologically active fragment thereof, to bind to or interact with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide to bind to or interact with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting the cellular

location of target molecule, detecting catalytic/enzymatic activity of the target molecule upon an appropriate substrate, detecting induction of a metabolite of the target molecule (*e.g.*, detecting the products of ATP hydrolysis, changes in intracellular K^+ levels) detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response (*i.e.*, membrane excitability, or cell growth, proliferation, differentiation, or apoptosis, sugar transport).

In yet another embodiment, an assay of the present invention is a cell-free assay in which a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or biologically active portion thereof is determined. Preferred biologically active portions of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides to be used in assays of the present invention include fragments which participate in interactions with non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt 3 molecules, *e.g.*, fragments with high surface probability scores (see, for example, Figures 2, 9, 13, 17, 21, 25, 29, 33, and 37). Binding of the test compound to the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or biologically active portion thereof with a known compound which binds 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, wherein determining the ability of the test compound to interact with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide comprises determining the ability of the test compound to preferentially bind to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be accomplished, for example, by determining the ability of the 8099, 46455, 54414, 53763,

67076, 67102, 44181, 67084FL, or 67084alt polypeptide to bind to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule by one of the methods described above for determining direct binding. Determining the ability of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide to bind to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be accomplished by determining the ability of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide to further modulate the activity of a downstream effector of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or biologically active portion thereof with a known compound which binds the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, wherein determining the ability of the test compound to interact with the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide comprises determining the ability of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide to preferentially bind to or modulate the activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule.

The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated proteins (*e.g.*, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt proteins or biologically active portions thereof). In the case of cell-free assays in which a membrane-bound form of an isolated protein is used it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-

dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, or interaction of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized micrometer plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or micrometer plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, substrate, or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with

8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or target molecules but which do not interfere with binding of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide to its target molecule can be derivatized to the wells of the plate, and unbound target or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or target molecule.

In another embodiment, modulators of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide in the cell is determined. The level of expression of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide in the presence of the candidate compound is compared to the level of expression of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide in the absence of the candidate compound. The candidate compound can then be identified as a modulator of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression based on this comparison. For example, when expression of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide expression. Alternatively, when expression of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide expression. The level of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or polypeptide.

In yet another aspect of the invention, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993)

Biotechniques 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt ("8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -binding proteins" or "8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -bp") and are involved in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity. Such 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -binding proteins are also likely to be involved in the propagation of signals by the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptides or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt targets as, for example, downstream elements of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -mediated signaling pathway. Alternatively, such 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -binding proteins are likely to be 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for cellular transformation and/or tumorigenesis.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent

identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt modulating agent, an antisense 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecule, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -specific antibody, or a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences, described herein, can be used to map the location of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt genes on a chromosome. The mapping of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences. Computer analysis of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene

corresponding to the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for

marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature,* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 or SEQ ID NO:4 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 or SEQ ID NO:6 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated

fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 or SEQ ID NO:4 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, or SEQ ID NO:13, having a length of at least 20 bases, preferably at least 30 bases.

The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide and/or nucleic acid expression as well as 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, nucleic acid expression or activity. For example, mutations in a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a

disorder characterized by or associated with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt in clinical trials.

These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid (*e.g.*, mRNA, or genomic DNA) that encodes 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide such that the presence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid is detected in the biological sample. In another aspect, the present invention provides a method for detecting the presence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity such that the presence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity is detected in the biological sample. A preferred agent for detecting 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or genomic DNA. The nucleic acid probe can be, for example, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:27, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, _____, _____, _____, or _____ , or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide is an antibody capable of binding to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA, polypeptide, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of PLTR polypeptide include introducing into a subject a labeled 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide; (ii) aberrant expression of a gene encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide; (iii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, wherein a wild-type form of the gene encodes a polypeptide with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity. "Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes, but is not limited to, expression at non-wild type levels (*e.g.*, over or under

expression); a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed (*e.g.*, increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage); a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene (*e.g.*, a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus).

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, mRNA, or genomic DNA, such that the presence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, mRNA or genomic DNA in the control sample with the presence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or mRNA in a biological sample; means for determining the amount of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt in the sample; and means for comparing the amount of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt

expression or activity. As used herein, the term "aberrant" includes a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity which deviates from the wild type 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity is intended to include the cases in which a mutation in the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene causes the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or a polypeptide which does not function in a wild-type fashion, *e.g.*, a protein which does not interact with or transport a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate, or one which interacts with or transports a non-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as deregulated cellular proliferation. For example, the term unwanted includes a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity which is undesirable in a subject.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide activity or nucleic acid expression, such as a as a cell growth, proliferation and/or differentiation disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide activity or nucleic acid expression, such as a cell growth, proliferation and/or differentiation disorder, a sugar trnasporter associated disorder, or a potassium channel associated disorder, as described herein. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity in which a test sample is obtained from a subject and 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity.

As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a sugar transporter-associated disorder, a potassium channel associated disorder, or phospholipid transporter-associated disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity in which a test sample is obtained and 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity).

The methods of the invention can also be used to detect genetic alterations in a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide activity or nucleic acid expression, such as a cell growth, proliferation and/or differentiation disorder. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -polypeptide, or the mis-expression of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene; 2) an addition of one or more nucleotides to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene; 3) a substitution of one or more nucleotides of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, 4) a chromosomal rearrangement of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene; 5) an alteration in the level of a messenger RNA transcript of a 8099, 46455, 54414, 53763, 67076, 67102,

44181, 67084FL, or 67084alt gene, 6) aberrant modification of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, 8) a non-wild type level of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -polypeptide, 9) allelic loss of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, and 10) inappropriate post-translational modification of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -polypeptide. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene (see Abravaya *et al.* (1995) *Nucleic Acids Res* .23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene under conditions such that hybridization and amplification of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene and detect mutations by comparing the sequence of the sample 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene include methods in which protection from

cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence, *e.g.*, a wild-type 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the

resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.,* in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene.

Furthermore, any cell type or tissue in which 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide (*e.g.*, the modulation of gene expression, cellular signaling, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity, phospholipid transporter activity, and/or cell growth, proliferation, differentiation, absorption, and/or secretion mechanisms) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene expression, polypeptide levels, or upregulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity, can be monitored in clinical trials of subjects exhibiting decreased 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene expression, polypeptide levels, or downregulated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene expression, polypeptide levels, or downregulate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity, can be monitored in clinical trials of subjects exhibiting increased 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene expression, polypeptide levels, or upregulated 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity. In such clinical trials, the expression or activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene, and preferably, other genes that have been implicated in, for example, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt , that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates 67076,

67102, 44181, 67084FL, or 67084alt activity (*e.g.*, identified in a screening assay as described herein) can be identified.

Thus, to study the effect of agents on phospholipid transporter-associated disorders (*e.g.*, disorders characterized by deregulated gene expression, cellular signaling, 67076, 67102, 44181, 67084FL, or 67084alt activity, phospholipid transporter activity, and/or cell growth, proliferation, differentiation, absorption, and/or secretion mechanisms), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of 67076, 67102, 44181, 67084FL, or 67084alt and other genes implicated in the transporter-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of polypeptide produced, by one of the methods as described herein, or by measuring the levels of activity of 67076, 67102, 44181, 67084FL, or 67084alt or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, mRNA, or genomic DNA in the pre-administration sample with the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt

expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity, *e.g.* a phospholipid transporter-associated disorder. "Treatment", as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, the symptoms of disease or disorder or the predisposition toward a disease or disorder. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecules of the present invention or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity, by administering to the subject a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt or an agent which modulates 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or at least one 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or

67084alt activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt aberrancy, for example, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt , 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt agonist or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell capable of expressing 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt with an agent that modulates one or more of the activities of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide activity associated with the cell, such that 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity in the cell is modulated. An agent that modulates 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide activity can be an agent as described herein, such as a nucleic acid or a polypeptide, a naturally-occurring target molecule of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide (*e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt substrate), a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibody, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt agonist or antagonist, a peptidomimetic of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activities. Examples of such stimulatory agents include active 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide and a nucleic acid molecule encoding 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt that has been introduced into the cell. In another embodiment, the agent inhibits one or more 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activities. Examples of

such inhibitory agents include antisense 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt nucleic acid molecules, anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt 3 antibodies, and 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity. In another embodiment, the method involves administering a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression or activity.

Stimulation of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity is desirable in situations in which 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt is abnormally downregulated and/or in which increased 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity is likely to have a beneficial effect. Likewise, inhibition of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity is desirable in situations in which 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt is abnormally upregulated and/or in which decreased 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity is likely to have a beneficial effect.

3. Pharmacogenomics

The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity (*e.g.*, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically), for example, disorders characterized by aberrant 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene expression, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity, membrane excitability or conductance, gene transcription, phospholipid transporter activity, cellular signaling, and/or cell growth, proliferation, differentiation, absorption, and/or secretion disorders associated with aberrant or unwanted

8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecule or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecule or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular

pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (*e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecule or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecule or 8099, 46455, 54414,

53763, 67076, 67102, 44181, 67084FL, or 67084alt modulator, such as a modulator identified by one of the exemplary screening assays described herein.

4. Use of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt Molecules as Surrogate Markers

The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (*e.g.*, with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (*e.g.*, early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (*e.g.*, an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecules of the invention are also useful as pharmacodynamic markers. As used herein, a "pharmacodynamic marker" is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the

drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug *in vivo*. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (*e.g.*, a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt antibodies may be employed in an immune-based detection system for a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide marker, or 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -specific radiolabeled probes may be used to detect a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am, J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, *e.g.*, McLeod *et al.* (1999) *Eur. J. Cancer* 35(12): 1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or polypeptide (*e.g.*, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt DNA may correlate 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt drug response. The use of

pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

VI. Electronic Apparatus Readable Media and Arrays

Electronic apparatus readable media comprising 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information is also provided. As used herein, "8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information" refers to any nucleotide and/or amino acid sequence information particular to the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt molecules of the present invention, including but not limited to full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequences, and the like. Moreover, information "related to" said 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information includes detection of the presence or absence of a sequence (*e.g.*, detection of expression of a sequence, fragment, polymorphism, etc.), determination of the level of a sequence (*e.g.*, detection of a level of expression, for example, a quantative detection), detection of a reactivity to a sequence (*e.g.*, detection of protein expression and/or levels, for example, using a sequence-specific antibody), and the like. As used herein, "electronic apparatus readable media" refers to any suitable medium for storing, holding or containing data or information that can be read and accessed directly by an electronic apparatus. Such media can include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as compact disc; electronic storage media such as RAM, ROM, EPROM, EEPROM and the like; general hard disks and hybrids of these categories such as magnetic/optical storage media. The medium is adapted or configured for having recorded thereon 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information of the present invention.

As used herein, the term "electronic apparatus" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the present invention include stand-alone computing apparatus; networks, including a local area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as a personal digital assistants (PDAs), cellular phone, pager and the like; and local and distributed processing systems.

As used herein, "recorded" refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate

manufactures comprising the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information.

A variety of software programs and formats can be used to store the sequence information on the electronic apparatus readable medium. For example, the sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like, as well as in other forms. Any number of dataprocessor structuring formats (*e.g.*, text file or database) may be employed in order to obtain or create a medium having recorded thereon the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information.

By providing 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information in readable form, one can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the sequence information in readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

The present invention therefore provides a medium for holding instructions for performing a method for determining whether a subject has a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder or a pre-disposition to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder, wherein the method comprises the steps of determining 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information associated with the subject and based on the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information, determining whether the subject has a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder or a pre-disposition to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder or a pre-disposition to a disease associated with a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt wherein the method comprises the steps of determining 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information associated with the subject, and based on the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information, determining whether the subject has a 8099, 46455, 54414, 53763,

67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder or a pre-disposition to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. The method may further comprise the step of receiving phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

The present invention also provides in a network, a method for determining whether a subject has a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder or a pre-disposition to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder associated with 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt , said method comprising the steps of receiving 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt and/or a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder, and based on one or more of the phenotypic information, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt information (*e.g.*, sequence information and/or information related thereto), and the acquired information, determining whether the subject has a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder or a pre-disposition to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a business method for determining whether a subject has a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder or a pre-disposition to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder, said method comprising the steps of receiving information related to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt and/or related to a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder, and based on one or more of the phenotypic information, the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt information, and the acquired information, determining whether the subject has a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder or a pre-disposition to a 8099, 46455, 54414, 53763, 67076, 67102, 44181,

67084FL, or 67084alt -associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The invention also includes an array comprising a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt sequence of the present invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt . This allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression *per se* and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder, progression of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder, and processes, such a cellular transformation associated with the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -associated disease or disorder.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (*e.g.*, acertaining the effect of 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt expression on the expression of other genes). This provides, for example, for a selection of alternate

molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (*e.g.*, including 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt) that could serve as a molecular target for diagnosis or therapeutic intervention.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF HUMAN 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, AND 67084alt cDNAs

In this example, the identification and characterization of the gene encoding human 8099, 46455, 54414, 53763, 67076, 67102, 44181, full length 67084 (67084FL), and 67084alt is described.

Isolation of the human 8099 and 46455 cDNAs

The invention is based, at least in part, on the discovery of a human gene encoding a novel polypeptide, referred to herein as human 8099. The entire sequence of the human clone 8099 was determined and found to contain an open reading frame termed human "8099." The nucleotide sequence of the human 8099 gene is set forth in Figures 1A-B and in the Sequence Listing as SEQ ID NO:1. The amino acid sequence of the human 8099 expression product is set forth in Figure 1 and in the Sequence Listing as SEQ ID NO: 2. The 8099 polypeptide comprises 617 amino acids. The coding region (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3. Clone 8099, comprising the coding region of human 8099, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

The invention is further based, at least in part, on the discovery of a human gene encoding a novel polypeptide, referred to herein as human 46455. The entire sequence of the human clone 46455 was determined and found to contain an open reading frame termed human "46455." The nucleotide sequence of the human 46455 gene is set forth in Figure 4 and in the Sequence Listing as SEQ ID NO:4. The amino acid sequence of the human 46455 expression product is set forth in Figures 8A-B and in the Sequence Listing as SEQ ID NO:5. The 46455 polypeptide comprises 528 amino acids. The coding region (open reading frame) of SEQ ID NO:4 is set forth as SEQ ID NO:6. Clone 46455, comprising the coding region of human 46455, was deposited with the American Type Culture Collection

(ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

Analysis of the Human 8099 and 46455 Molecules

A search using the polypeptide sequence of SEQ ID NO:2 was performed against the HMM database in PFAM (Figures 3A-B) resulting in the identification of a sugar transporter family domain in the amino acid sequence of human 8099 at about residues 43-564 of SEQ ID NO:2 (score = 318.2), a potential FecCD family domain in the amino acid sequence of human 8099 at about residues 26-227 of SEQ ID NO:2 (score = -218.2), and a potential monocarboxylate transporter domain in the amino acid sequence of human 8099 at about residues 29-567 of SEQ ID NO:2 (score = -235.8).

The amino acid sequence of human 8099 was analyzed using the program PSORT (available through the Prosite website) to predict the localization of the proteins within the cell. This program assesses the presence of different targeting and localization amino acid sequences within the query sequence. The results of this analysis show that human 8099 may be localized to the endoplasmic reticulum or mitochondria.

Searches of the amino acid sequence of human 8099 were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human 8099 of a number of potential N-glycosylation sites at about amino acid residues 371-374, 383-386, 396-399, 401-404 of SEQ ID NO:2, a number of potential protein kinase C phosphorylation sites at about amino acid residues 220-222, 256-258, 403-405 of SEQ ID NO:2, a number of potential casein kinase II phosphorylation sites at about amino acid residues 18-21, 75-78, 169-172, 246-249, 256-259, 264-267, 385-388, 403-406, 443-446, 520-523 of SEQ ID NO:2, a number of potential N-myristoylation sites at about amino acid residues 51-56, 59-64, 89-94, 141-146, 165-170, 178-183, 207-212, 228-233, 395-400, 441-446, and 493-498 of SEQ ID NO:2, a potential amidation site at about amino acid residues104-107 of SEQ ID NO:2, a potential leucine zipper motif at about amino acid residues 112-133 of SEQ ID NO:2, and potential sugar transport protein signature 1 domain at about amino acid residues 97-114 of SEQ ID NO:2.

A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:2 was also performed, predicting thirteen transmembrane domains in the amino acid sequence of human 8099 (SEQ ID NO:2) at about residues 32-49, 58-74, 81-101, 109-130, 138-156, 165-184, 198-217, 279-301, 315-338, 346-364, 463-487, 499-521, and 529-549. Further analysis of the amino acid sequence of SEQ ID NO:2 (*e.g.*, alignment with, for example, known *E. coli* sugar symporter proteins and a known human facilitative glucose transporter protein) showed that the second transmembrane domain at about amino acid residues 58-74 of SEQ ID NO:2 is not utilized, resulting in the presence of twelve transmembrane domains in the amino acid sequence of human 8099 (SEQ ID NO:2).

A search of the amino acid sequence of human 8099 was also performed against the ProDom database resulting in the identification of several transmembrane domains, a glycosyltransferase domain, and a sugar transport domain in the amino acid sequence of SEQ ID NO:2.

The human 8099 amino acid sequence was aligned with the amino acid sequence of the galactose-proton symporter GALP from *E. coli* using the CLUSTAL W (1.74) multiple sequence alignment program. The results of the alignment are set forth in Figure 4. The human 8099 amino acid sequence was also aligned with the amino acid sequence of the arabinose-proton symporter ARAE from *E. coli* using the CLUSTAL W (1.74) multiple sequence alignment program. The results of the alignment are set forth in Figure 5. The human 8099 amino acid sequence was also aligned with the amino acid sequence of the facilitative glucose transporter GLUT8 from *Homo sapiens* using the CLUSTAL W (1.74) multiple sequence alignment program. The results of the alignment are set forth in Figure 7. Based on its homology to GLUT8, 8099 is also referred to herein as "GLUT8 homologue" or "GLUT8h" and can be used interchangeably throughout.

A search using the polypeptide sequence of human 46455 (SEQ ID NO:5) was performed against the HMM database in PFAM (Figures 10A-C) resulting in the identification of a sugar transporter family domain in the amino acid sequence of human 46455 at about residues 58-469 of SEQ ID NO:5 (score = -63.4), a potential sodium:galactoside symporter family domain in the amino acid sequence of human 46455 at about residues 212-505 of SEQ ID NO:5 (score = -121.2), and a potential monocarboxylate transporter domain in the amino acid sequence of human 46455 at about residues 60-473 of SEQ ID NO:5 (score = -208.2).

The amino acid sequence of human 46455 was analyzed using the program PSORT to predict the localization of the proteins within the cell. This program assesses the presence of different targeting and localization amino acid sequences within the query sequence. The results of this analysis show that human 46455 may be localized to the endoplasmic reticulum, mitochondria, nucleus or secretory vesicles.

Searches of the amino acid sequence of human 46455 were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human 46455 of a potential N-glycosylation site at about amino acid residues 199-202 of SEQ ID NO:5, a potential cAMP- and cGMP-dependent protein kinase C phosphorylation site at about amino acid residues 414-417 of SEQ ID NO:5, a number of potential protein kinase C phosphorylation sites at about amino acid residues 344-346, 413-415, 442-444, and 518-520 of SEQ ID NO:5, a number of potential casein kinase II phosphorylation sites at about amino acid residues 11-14, 943-946, 959-962, 983-986, 1074-1077, 1108-1111, and 1112-1115 of SEQ ID NO:5, a number of potential N-myristoylation sites at about amino acid residues 89-94, 106-111, 288-293, 679-684, 767-772, 847-852, and

933-938 of SEQ ID NO:5, an amidation site at about amino acid residues 223-226 of SEQ ID NO:5, and an ATP/GTP-binding site motif A (P-loop) at about amino acid residues 1008-1015 of SEQ ID NO:5.

A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:5 was also performed, predicting eleven transmembrane domains in the amino acid sequence of human 46455 (SEQ ID NO:5) at about residues 98-118, 126-145, 165-181, 188-205, 218-238, 273-294, 323-341, 357-377, 386-410, 423-441, and 462-485. Further analysis of the amino acid sequence of SEQ ID NO:5 (*e.g.*, analysis of the hydropathy plot set forth in Figure 9) resulted in the identification of a twelfth transmembrane domain at about amino acid residues 58-74 of SEQ ID NO:5.

A search of the amino acid sequence of human 46455 was also performed against the ProDom database resulting in the identification of a transmembrane efflux domain in the amino acid sequence of SEQ ID NO:5.

The human 46455 amino acid sequence was aligned with the amino acid sequence of Z92825 from *C. elegans* using the CLUSTAL W (1.74) multiple sequence alignment program. The results of the alignment are set forth in Figure 11.

Isolation of the human 54414 and 53763 cDNA

The invention is based, at least in part, on the discovery of genes encoding novel members of the potassium channel family. The entire sequence of human clone Fbh54414 was determined and found to contain an open reading frame termed human "54414". The entire sequence of human clone Fbh53763 was determined and found to contain an open reading frame termed human "53763".

The nucleotide sequence encoding the human 54414 is shown in Figures 12A-D and is set forth as SEQ ID NO:7. The protein encoded by this nucleic acid comprises about 1118 amino acids and has the amino acid sequence shown in Figures 12A-D and set forth as SEQ ID NO:8. The coding region (open reading frame) of SEQ ID NO:7 is set forth as SEQ ID NO:9. Clone Fbh54414, comprising the coding region of human 54414, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on ____, and assigned Accession No. _____.

The nucleotide sequence encoding the human 53763 is shown in Figures 16A-C and is set forth as SEQ ID NO:10. The protein encoded by this nucleic acid comprises about 638 amino acids and has the amino acid sequence shown in Figures 16A-C and set forth as SEQ ID NO:11. The coding region (open reading frame) of SEQ ID NO:10 is set forth as SEQ ID NO:12. Clone Fbh53763, comprising the coding region of human 53763, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _, and assigned Accession No. _____.

Analysis of the human 54414 and 53763 Molecules

The amino acid sequences of human 54414 was analyzed using the program PSORT to predict the localization of the proteins within the cell. The results of the analyses show that human 54414 may be localized to the endoplasmic reticulum, the nucleus, secretory vesicles, or the mitochondria.

Analysis of the amino acid sequences of human 54414 was performed using MEMSAT. The amino acid sequence of human 54414 was also compared to the amino acid sequences of known potassium transporters (Figures 15A-B). This analysis resulted in the identification of six possible transmembrane domains in the amino acid sequence of human 54414 at residues 64-83, 104-127, 135-153, 161-173, 199-217, and 257-274 of SEQ ID NO:8 (Figure 13).

Searches of the amino acid sequences of human 54414 were performed against the HMM database (Figure 14). These searches resulted in the identification of an "ion transport protein domain", at about residues 104-277 of SEQ ID NO:8 (score= 62.4).

Searches of the amino acid sequence of human 54414 were further performed against the Prosite™ database. These searches resulted in the identification of several possible N-glycosylation sites at about amino acids residues 66-69, 99-102, 290-293, 545-548, 554-557, 573-576, 981-984, and 1106-1109 of SEQ ID NO:8, several possible cAMP- and cGMP-dependent protein kinase phosphorylation sites at about amino acids residues 625-628, 994-997, 1002-1005, and 1100-1103 of SEQ ID NO:8, several possible protein kinase C phosphorylation sites at about amino acid residues 43-45, 59-61, 68-70, 126-128, 158-160, 254-256, 298-300, 308-310, 354-356, 385-387, 464-466, 605-607, 903-905, 939-941, 947-949, 1005-1007, 1012-1014, 1030-1032, and 1099-1101 of SEQ ID NO:8, several possible casein kinase II phosphorylation sites at about amino acid residues 43-46, 115-118, 338-341, 386-389, 393-396, 485-488, 556-559, 651-654, 655-658, 663-666, 784-787, 837-840, 867-870, 907-910, 926-929, 943-946, 959-962, 983-986, 1074-1077, 1108-1111, and 1112 -1115 of SEQ ID NO:8, several possible N-myristoylation sites at about amino acid residues 89-94, 106-111, 288-293, 679-684, 767-772, 847-852, and 933-938 of SEQ ID NO:8, a possible amidation site at about amino acid residues 223-226 of SEQ ID NO:8, and a possible ATP/GTP-binding site motif A (P-loop) at about amino acid residues 1008-1015 of SEQ ID NO:8.

The amino acid sequence of human 53763 was analyzed using the program PSORT to predict the localization of the proteins within the cell. The results of the analyses further show that human 53763 may be localized to the endoplasmic reticulum, the mitochondria, or the nucleus.

Analysis of the amino acid sequences of human 53763 was performed using MEMSAT. The amino acid sequence of human 53763 was also compared to the amino acid sequences of known potassium transporters (Figure 19). This analysis resulted in the

identification of six possible transmembrane domains in the amino acid sequence of human 53763 at residues 230-248, 287-303, 314-335, 346-368, 382-402, and 451-473 of SEQ ID NO:11 (Figure 17).

Searches of the amino acid sequence of human 53763 were also performed against the HMM database (Figures 18A-B). These searches resulted in the identification of a "NADH-ubiquinone/plastoquinone oxidoreductase domain" at about residues 317-467 of SEQ ID NO:11 (score= -81.7), an "ion transport protein domain" at about residues 281-472 of SEQ ID NO:11 (score = 116.9), and a "K^+ channel tetramerisation domain" at about residues 8-156 of SEQ ID NO:11 (score = 156.7).

Searches of the amino acid sequence of human 53763 were also performed against the Prosite™ database. These searches resulted in the identification in the amino acid sequence of human 53763 a number of potential N-glycosylation sites at amino acid residues 84-84, 259-262, 266-269, 518-521, and 536-539 of SEQ ID NO:11, a potential cAMP- and cGMP-dependent protein kinase phosphorylation site at amino acid residues 561-564 of SEQ ID NO:11, protein kinase C phosphorylation sites at amino acid residues 21-23, 25-27, 86-88, 120-122, 155-157, 205-207, 224-226, 336-338, 374-376, and 564-566 of SEQ ID NO:11, casein kinase II phosphorylation sites at amino acid residues 17-20, 49-52, 146-149, 283-286, 378-381, 414-417, 520-523, 541-544, 546-549, 553-556, 564-567, and 579-582 of SEQ ID NO:11, and N-myristoylation sites at amino acid residues 31-36, 76-81, 83-88, 89-94, 142-147, 176-181, 191-196, 199-204, 407-412, 450-455, 477-482, 590-595, and 606-611 of SEQ ID NO:11.

Searches of the amino acid sequences of human 54414 and human 53763 were also performed against the ProDom database. A potassium ionic calcium activated domain and two potassium ionic subunits were identified in the amino acid sequence of 54414 (SEQ ID NO:8). Several transmembrane domains and transport family domains were identified in the the amino acid sequence of 53763 (SEQ ID NO:11).

The amino acid sequences of human 54414 and human 53763 were further analyzed for the presence of a "pore domain", also known as a "P-region domain". A pore domain was identified in the amino acid sequence of human 54414 at about residues 229-250 of SEQ ID NO:8. A pore domain was identified in the amino acid sequence of human 53763 at about residues 426-441 of SEQ ID NO:11.

The amino acid sequences of human 54414 and human 53763 were also analyzed for the presence of a "potassium channel signature sequence motif" (see Joiner, W. J. *et al.* (1998) *Nat. Neurosci.* 1:462-469 and references cited therein). A potassium channel signature sequence motif was identified in the amino acid sequence of human 54414 at about residues 239-246 of SEQ ID NO:8. A potassium channel signature sequence motif was identified in the amino acid sequence of human 53763 at about residues 436-441 of SEQ ID NO:11.

The amino acid sequence of human 53763 was also analyzed for the presence of a "voltage sensor motif". A voltage sensor motif was identified in the amino acid sequence of human 53763 at about residues 348-363 of SEQ ID NO:11. Positively charged amino acid residues in the voltage sensor motif were identified about residues 348, 351, 354, 357, 360, and 363 of SEQ ID NO:5.

Isolation of the human 67076, 67102, 44181, 67084FL, or 67084alt cDNAs

The invention is based, at least in part, on the discovery of a human gene encoding novel polypeptides, referred to herein as human 67076, 67102, 44181, 67084FL, and 67084alt. The entire sequence of the human clone 67076 was determined and found to contain an open reading frame termed human "67076." The nucleotide sequence of the human 67076 gene is set forth in Figures 20A-E and in the Sequence Listing as SEQ ID NO:13. The amino acid sequence of the human 67076 expression product is set forth in Figures 20A-E and in the Sequence Listing as SEQ ID NO:14. The 67076 polypeptide comprises 1129 amino acids. The coding region (open reading frame) of SEQ ID NO:13 is set forth as SEQ ID NO:15. Clone 67076, comprising the coding region of human 67076, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

The entire sequence of the human clone 67102 as determined and found to contain an open reading frame termed human "67102." The nucleotide sequence of the human gene is set forth in Figures 24A-E and in the Sequence Listing as SEQ ID NO:16. The amino acid sequence of the human 67102 expression product is set forth in Figures 24A-E and in the Sequence Listing as SEQ ID NO:17. The 67102 polypeptide comprises 1426 amino acids. The coding region (open reading frame) of SEQ ID NO:16 is set forth as SEQ ID NO:18. Clone 67102, comprising the coding region of human 67102, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

The entire sequence of the human clone 44181 was determined and found to contain an open reading frame termed human "44181." The nucleotide sequence of the human 44181 gene is set forth in Figures 28A-E and in the Sequence Listing as SEQ ID NO:19. The amino acid sequence of the human 44181 expression product is set forth in Figure 7A-E and in the Sequence Listing as SEQ ID NO:20. The 44181 polypeptide comprises 1177 amino acids. The coding region (open reading frame) of SEQ ID NO:19 is set forth as SEQ ID NO:21. Clone 44181, comprising the coding region of human 44181, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

The entire sequence of the human clone 67084FL was determined and found to contain an open reading frame termed human "67084FL." The nucleotide sequence of the

human 67084FL gene is set forth in Figures 32A-D and in the Sequence Listing as SEQ ID NO:22. The amino acid sequence of the human 67084FL expression product is set forth in Figures 32A-D and in the Sequence Listing as SEQ ID NO:23. The 67084FL polypeptide comprises 1084 amino acids. The coding region (open reading frame) of SEQ ID NO:22 is set forth as SEQ ID NO:24. Clone 67084FL, comprising the coding region of human 67084FL, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

The entire sequence of the human clone 67084alt was determined and found to contain an open reading frame termed human "67084alt." The nucleotide sequence of the human 67084alt gene is set forth in Figures 36A-D and in the Sequence Listing as SEQ ID NO:25. The amino acid sequence of the human 67084alt expression product is set forth in Figures 36A-D and in the Sequence Listing as SEQ ID NO:26. The 67084alt polypeptide comprises 1095 amino acids. The coding region (open reading frame) of SEQ ID NO:25 is set forth as SEQ ID NO:27. Clone 67084alt, comprising the coding region of human 67084alt, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

Analysis of the Human 67076, 67102, 44181, 67084FL, or 67084alt Molecules

The amino acid sequences of human 67076, 67102, 44181, 67084FL, or 67084alt were analyzed for the presence of sequence motifs specific for P-type ATPases (as defined in Tang, X. et al. (1996) *Science* 272:1495-1497 and Fagan, M. J. and Saier, M. H. (1994) *J. Mol. Evol.* 38:57).

These analyses resulted in the identification of a P-type ATPase sequence I motif in the amino acid sequence of human 67076 at residues 173-181 of SEQ ID NO:14, in the amino acid sequence of human 67102 at residues 208-216 of SEQ ID NO:17, in the amino acid sequence of human 44181 at residues 173-181 of SEQ ID NO:20, in the amino acid sequence of human 67084FL at residues 213-221 of SEQ ID NO:23, and in the amino acid sequence of human 67084alt at residues 213-221 of SEQ ID NO:26.

These analyses also resulted in the identification of a P-type ATPase sequence 2 motif in the amino acid sequence of human 67076 at residues 406-415 of SEQ ID NO:14, in the amino acid sequence of human 67102 at residues 435-444 of SEQ ID NO:17, in the amino acid sequence of human 44181 at residues 404-413 of SEQ ID NO:20, in the amino acid sequence of human 67084FL at residues 413-422 of SEQ ID NO:23, and in the amino acid sequence of human 67084alt at residues 413-422 of SEQ ID NO:26.

These analyses further resulted in the identification of a P-type ATPase sequence 3 motif in the amino acid sequence of human 67076 at residues 813-824 of SEQ ID NO:14, in

the amino acid sequence of human 67102 at residues 1054-1064 of SEQ ID NO:17, in the amino acid sequence of human 44181 at residues 819-829 of SEQ ID NO:20, in the amino acid sequence of human 67084FL at residues 820-830 of SEQ ID NO:23, and in the amino acid sequence of human 67084alt at residues 820-830 of SEQ ID NO:26.

The amino acid sequences of human 67076, 67102, 44181, 67084FL, and 67084alt were also analyzed for the presence of phospholipid transporter specific amino acid residues (as defined in Tang, X. et al. (1996) *Science* 272:1495-1497). These analyses also resulted in the identification of phospholipid transporter specific amino acid residues in the amino acid sequence of human 67076 at residues 174, 177, 407, 813, 823, and 824 of SEQ ID NO:14. These analyses resulted in the identification of phospholipid transporter specific amino acid residues 208, 209, 212, 436, 1054, 1063, and 1064 in the amino acid sequence of human 67102 at residues of SEQ ID NO:17. These analyses further resulted in the identification of phospholipid transporter specific amino acid residues 174, 177, 405, 819, 928, and 929 in the amino acid sequence of human 44181 at residues of SEQ ID NO:20. These analyses further resulted in the identification of phospholipid transporter specific amino acid residues 214, 217, 820, 829, and 830 in the amino acid sequence of human 67084FL at residues of SEQ ID NO:23. These analyses still further resulted in the identification of phospholipid transporter specific amino acid residues 214, 217, 820, 829, and 830 in the amino acid sequence of human 67084alt at residues of SEQ ID NO:26.

The amino acid sequences of human 67076, 67102, 44181, 67084FL, and 67084alt were also analyzed for the presence of extramembrane domains. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 67076 at residues 105-291 of SEQ ID NO:14. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 67076 at residues 366-872 of SEQ ID NO:14. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 67102 at residues 141-321 of SEQ ID NO:17. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 67102 at residues 391-581 of SEQ ID NO:17. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 44181 at residues 105-289 of SEQ ID NO:20. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 44181 at residues 364-877 of SEQ ID NO:20. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 67084FL at residues 145-330 of SEQ ID NO:23. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 67084FL at residues 380-886 of SEQ ID NO:23. An N-terminal large extramembrane domain was identified in the amino acid sequence of human 67084alt at residues 145-330 of SEQ ID NO:26. A C-terminal large extramembrane domain was identified in the amino acid sequence of human 67084alt at residues 380-886 of SEQ ID NO:26.

The amino acid sequence of human 67076 was analyzed using the program PSORT to predict the localization of the proteins within the cell. This program assesses the presence of different targeting and localization amino acid sequences within the query sequence. The results of this analysis predict that human 67076 may be localized to the endoplasmic reticulum.

Searches of the amino acid sequence of human 67076 were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human 67076 of a number of potential N-glycosylation sites at amino acid residues121-124, 392-395, 761-764, 992-995, and 1098-1101 of SEQ ID NO:14, a number of potential cAMP-and cGMP-dependent protein kinase phosphorylation sites at amino acid residues135-138, 545-548, 1091-1094, and 1102-1105 of SEQ ID NO:14, a number of potential protein kinase C phosphorylation sites at amino acid residues 47-49, 138-140, 204-206, 250-252, 254-256, 278-280, 308-310, 328-330, 334-336, 408-410, 680-682, 701-703, 708-710, 733-735, 736-738, 1008-1010, 1094-1096, 1100-1102, 1109-1111, and 1113-1115 of SEQ ID NO:14, a number of casein kinase II phosphorylation sites at amino acid residues 30-33, 264-267, 282-285, 328-331, 413-416, 442-445, 449-452, 494-497, 646-649, 693-696, 704-707, 762-765, 813-816, 924-927, 982-985, and 1121-1124 of SEQ ID NO:14, a number of potential tyrosine kinase phosphorylation sites at amino acid residues 252-258, 739-747 of SEQ ID NO:14, a number of N-myristoylation sites at amino acid residues 388-393, 440-445, 482-487, 514-519, 564-569, 753-758, and 807-812 of SEQ ID NO:14, an ATP/GTP-binding site motif (P-loop) at amino acid residues 271-278 of SEQ ID NO:14, and an E1-E2 ATPases phosphorylation site at amino acid residues 409-415 of SEQ ID NO:14.

A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:14 was also performed, predicting three potential transmembrane domains in the amino acid sequence of human 67076 (SEQ ID NO:14). However, a structural, hydrophobicity, and antigenicity analysis (Figure 21) resulted in the identification of ten transmembrane domains. Accordingly, the 67076 protein of SEQ ID NO:14 is predicted to have at least ten transmembrane domains, identified as transmembrane (TM) domains 1 through 10, at about residues 57-77, 84-105, 292-313, 345-365, 863-883, 905-926, 956-977, 989-1009, 1021-1041, and 1060-1087.

A search using the polypeptide sequence of SEQ ID NO:14 was performed against the HMM database in PFAM resulting in the identification of a potential hydrolase domain in the amino acid sequence of human 67076 at about residues 403-837 of SEQ ID NO:14 (score = 12.7).

A search of the amino acid sequence of human 67076 was also performed against the ProDom database resulting in the identification of several hydrolase domains and phosphorylation domains in the amino acid sequence of 67076 (SEQ ID NO:14).

The amino acid sequence of human 67102 was analyzed using the program PSORT. The results of this analysis predict that human 67102 may be localized to the endoplasmic reticulum.

Searches of the amino acid sequence of human 67102 were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human 67102 of a number of potential N-glycosylation sites at amino acid residues 29-32, 303-306, 1365-1368, and 1397-1400 of SEQ ID NO:17, a glycosaminoglycan attachment site at amino acid residues 526-529 of SEQ ID NO:17, a number of potential cAMP- and cGMP-dependent protein kinase phosphorylation sites at amino acid residues 38-41, 451-545, 635-638, and 777-780 of SEQ ID NO:17, a number of protein kinase C phosphorylation sites at amino acid residues 47-49, 78-80, 161-163, 240-242, 262-264, 280-282, 437-439, 500-502, 563-565, 633-635, 644-646, 695-697, 743-745, 774-776, 827-829, 1000-1002, 1360-1362, and 1371-1373 of SEQ ID NO:17, a number of potential casein kinase II phosphorylation sites at amino acid residues 20-23, 161-164, 176-179, 184-187, 199-202, 210-213, 232-235, 241-244, 262-265, 312-315, 345-348, 405-408, 442-445, 471-474, 477-480, 543-546, 621-624, 644-647, 670-673, 693-696, 727-730, 850-853, 866-869, 892-895, 977-980, 1074-1077, 1141-1144, 1199-1202, 1221-1224, 1339-1342, 1399-1402, and 1403-1406 of SEQ ID NO:17, two tyrosine kinase phosphorylation sites at amino acid residues 21-28 and 847-854 of SEQ ID NO:17, a number of potential N-myristoylation sites at amino acid residues 69-74, 341-346, 488-493, 510-515, 519-524, 525-530, 651-656, 703-708, 714-719, 901-906, 955-960, 992-997, 1070-1075, 1139-1144, 1229-1234, and 1261-1266 of SEQ ID NO:17, two potential amidation sites at amino acid residues 36-39 and 1371-1374 of SEQ ID NO:17, two ATP/GTP-binding site motif A (P-loop) at amino acid residues 996-1003 and 1364-1371, an E1-E2 ATPases phosphorylation site at amino acid residues 438-444 of SEQ ID NO:17, and a prokaryotic membrane lipoprotein lipid attachment site at amino acid residues 26-36 of SEQ ID NO:17.

A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:17 was also performed, predicting ten potential transmembrane domains in the amino acid sequence of human 67102 (SEQ ID NO:17) at about residues 98-115, 122-140, 322-344, 366-390, 582-601, 752-770, 1145-1166, 1225-1246, 1253-1276, and 1298-1317.

A search using the polypeptide sequence of SEQ ID NO:17 was performed against the HMM database in PFAM resulting in the identification of a potential hydrolase domain in the amino acid sequence of human 67102 at about residues 432-1077 of SEQ ID NO:17 (score =1.5), and the identification of a potential DUF6 domain in the amino acid sequence of human 67102 at about residues 1127-1271 of SEQ ID NO:17 (score = -24.6).

A search of the amino acid sequence of human 67102 was also performed against the ProDom database resulting in the identification of several hydrolase domains and phosphorylation domains in the amino acid sequence of 667102 (SEQ ID NO:17).

The amino acid sequence of human 44181 was analyzed using the program PSORT. The results of this analysis predict that human 44181 may be localized to the endoplasmic reticulum.

Searches of the amino acid sequence of human 44181 were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human 44181 of a number of potential N-glycosylation sites at amino acid residues 331-334, 390-393, 449-452, 461-464, 477-480, 786-789, and 998-1001 of SEQ ID NO:20, a number of potential cAMP- and cGMP-dependent protein kinase phosphorylation sites at amino acid residues 577-580, 633-636, and 750-753 of SEQ ID NO:20, a number of protein kinase C phosphorylation sites at amino acid residues 46-48, 163-165, 276-278, 332-334, 406-408, 470-472, 574-576, 636-638, 957-959, 1014-1016, and 1102-1104 of SEQ ID NO:20, a number of potential casein kinase C phosphorylation sites at amino acid residues 115-118, 262-265, 280-283, 411-414, 473-476, 520-523, 527-530, 636-639, 678-681, 737-740, 906-909, 929-932, 1100-1103, 1154-1157, and 1170-1173 of SEQ ID NO:20, a potential tyrosine kinase phosphorylation site at amino acid residues 316-322 of SEQ ID NO:20, a number of potential N-myristoylation sites at amino acid residues 131-136, 596-601, 766-771, and 993-998 of SEQ ID NO:20, and an E1-E2 ATPases phosphorylation site at amino acid residues 407-413 of SEQ ID NO:20.

A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:20 was also performed, predicting three potential transmembrane domains in the amino acid sequence of human 44181 (SEQ ID NO:20). However, a structural, hydrophobicity, and antigenicity analysis (Figure 29) resulted in the identification of ten transmembrane domains. Accordingly, the 44181 protein (SEQ ID NO:20) is predicted to have at least ten transmembrane domains, which are identified as transmembrane (TM) domains 1 through 10, at about residues 56-72, 87-103, 290-311, 343-363, 878-898, 911-931, 961-982, 995-1015, 1027-1047, and 1062-1086.

A search using the polypeptide sequence of SEQ ID NO:20 was performed against the HMM database in PFAM resulting in the identification of a potential E1-E2 ATPase domain in the amino acid sequence of human 44181 at about residues 126-164 of SEQ ID NO:20 (score =8.6), the identification of a potential DUF132 domain in the amino acid sequence of human 44181 at about residues 579-719 of SEQ ID NO:20 (score = -72.9), and the identification of a potential hydrolase domain in the amino acid sequence of human 44181 at about residues 401-842 of SEQ ID NO:20 (score = 42.8).

A search of the amino acid sequence of human 44181 was also performed against the ProDom database A search of the amino acid sequence of human 44181 was also performed against the ProDom database resulting in the identification of several hydrolase domains and phosphorylation domains in the amino acid sequence of 44181 (SEQ ID NO:20).

A Clustal W (1.74) alignment of the amino acid sequence of human 44181 (SEQ ID NO:20) and human potential phospholipid-transporting ATPase IR (ATIR; GenBank Accession No.:Q9Y2G3) revealed some sequence homology between 44181 and Accession No.:Q9Y2G3.

The amino acid sequence of human 67084FL was analyzed using the program PSORT. The results of this analysis predict that human 67084FL may be localized to the endoplasmic reticulum.

Searches of the amino acid sequence of human 67084FL were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human 67084FL of a number of potential N-glycosylation sites at amino acid residues 310-313, 464-467, and 529-532 of SEQ ID NO:23, a potential cAMP- and cGMP-dependent protein kinase phosphorylation site at amino acid residues 1071-1074 of SEQ ID NO:23, a number of protein kinase C phosphorylation sites 82-84, 168-170, 204-206, 301-303, 371-373, 415-417, 486-488, 585-587, 666-668, 744-746, 800-802, 813-815, 872-874, 957-959, and 1009-1011 of SEQ ID NO:23, a number of potential casein kinase II phosphorylation sites at amio acid residues 265-268, 301-304, 402-405, 422-425, 535-538, 596-599, 661-664, 686-689, and 745-748 of SEQ ID NO:23, a tyrosine kinase phosphorylation site at amino acid residues 813-816 of SEQ ID NO:23, a number of potential N-myristoylation sites at amino acid residues 292-297, 462-467, 568-573, 606-611, 824-829, 887-892, and 1026-1031 of SEQ ID NO:23, a potential amidation site at amino acid residues 813-816 of SEQ ID NO:23, a prokaryotic membrane lipoprotein lipid attachment site at amino acid residues 105-115, a leucine zipper pattern at amino acid residues 325-346, and an E1-E2 ATPases phosphorylation site at amino acid residues 416-422 of SEQ ID NO:23.

A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:23 was also performed, predicting nine potential transmembrane domains in the amino acid sequence of human 67084FL (SEQ ID NO:23). However, a structural, hydrophobicity, and antigenicity analysis (Figure 33) resulted in the identification of ten transmembrane domains. Accordingly, the 67084FL protein of SEQ ID NO:23 is predicted to have at least ten transmembrane domains, which are identified as transmembrane (TM) domains 1 through 10, at about residues 104-120, 124-144, 331-350, 357-374, 887-903, 912-931, 961-983, 990-1008, 1015-1035, and 1043-1067.

A search using the polypeptide sequence of SEQ ID NO:23 was performed against the HMM database in PFAM resulting in the identification of two potential E1-E2 ATPase in the amino acid sequence of human 67084FL at about residues 171-199 of SEQ ID NO:23 (score = 3.0) and 277-305 of SEQ ID NO:23 (score = 13.0), and a hydrolase domain at about residues 410-843 of of SEQ ID NO:23 (score = 19.2).

A search of the amino acid sequence of human 67084FL was also performed against the ProDom database resulting in the identification of several hydrolase domains, phosphorylation domains, and ATPase domains in the amino acid sequence of 67084FL (SEQ ID NO:23).

A Clustal W (1.74) alignment of the amino acid sequence of human 67084FL (SEQ ID NO:23) and human membrane transport protein (MTRP-1; GenBank Accession No.:Y71056, International Publicaiton No. WO 2000/26245-A2) revealed some sequence homology between 67084FL and Accession No.: Y71056.

The amino acid sequence of human 67084alt was analyzed using the program PSORT. The results of this analysis predict that human 67084alt may be localized to the endoplasmic reticulum.

Searches of the amino acid sequence of human 67084alt were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human 67084alt of a number of potential N-glycosylation sites at amino acid residues 310-313, 464-467, and 529-532 of SEQ ID NO:26, a potential cAMP- and cGMP-dependent protein kinase phosphorylation site at amino acid residues 1083-1086, a number of protein kinase C phosphorylation sites at amino acid residues 82-84, 168-170, 204-2-6, 301-303, 371-373, 415-417, 486-488, 585-587, 666-668, 744-746, 800-802, 813-815, 872-874, 957-959, and 1009-1011 of SEQ ID NO:26, a number of potential casein kinase II phosphorylation sites at amino acid residues 265-268, 301-304, 402-405, 422-445, 535-538, 596-599, 661-664, 686-689, and 745-748 of SEQ ID NO:26, a tyrosine kinase phosphorylation site at amino acid residues 641-648, a number of potential N-myristoylation sites at amino acid residues 292-297, 462-467, 568-573, 606-611, 824-829, 887-892, and 1026-1031 of SEQ ID NO:26, a potential amidation site at amino acid residues 813-816 of SEQ ID NO:26, a potential prokaryotic membrane lipoprotein lipid attachment site at amino acid residues 105-115 of SEQ ID NO:26, a leucine zipper pattern at amino acid residues 325-346 of SEQ ID NO:26, and an E1-E2 ATPases phosphorylation site at amino acid residues 416-422 of SEQ ID NO:26.

A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:26 was also performed, predicting nine potential transmembrane domains in the amino acid sequence of human 67084alt (SEQ ID NO:26). However, a structural, hydrophobicity, and antigenicity analysis (Figure 37) resulted in the identification of ten transmembrane domains.

Accordingly, the 67084alt protein of SEQ ID NO:26 is predicted to have at least ten transmembrane domains, which are identified as transmembrane (TM) domains 1 through 10, at about residues 104-120, 124-144, 331-350, 357-374, 887-903, 912-931, 961-983, 990-1008, 1015-1035, and 1043-1067.

A search using the polypeptide sequence of SEQ ID NO:26 was performed against the HMM database in PFAM resulting in the identification of two potential E1-E2 ATPase in the amino acid sequence of human 67084alt at about residues 42-70 of SEQ ID NO:26 (score = 3.0) and 105-133 of SEQ ID NO:26 (score = 13.0), and a potential hydrolase domain at about amino acid residues 410-843 of SEQ ID NO:26 (score = 19.2).

A search of the amino acid sequence of human 67084alt was also performed against the ProDom database resulting in the identification of several hydrolase domains, phosphorylation domains, and ATPase domains in the amino acid sequence of 67084alt (SEQ ID NO:26).

A Clustal W (1.74) alignment of the amino acid sequence of human 67084alt (SEQ ID NO:14) and human membrane transport protein (MTRP-1; GenBank Accession No.:Y71056, International Publicaiton No. WO 2000/26245-A2) revealed some sequence homology between 67084alt and Accession No.: Y71056.

EXAMPLE 2: TISSUE EXPRESSION OF HUMAN 8099, 46455, 54414, 53763, 67076, 67102, 44181, full length 67084 (67084FL), and 67084alt mRNA

Tissue Distribution of Human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, and 67084alt mRNA Using Taqman™ Analysis

This example describes the tissue distribution of human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt mRNA in a variety of cells and tissues, as determined using the TaqMan™ procedure. The Taqman™ procedure is a quantitative, reverse transcription PCR-based approach for detecting mRNA. The RT-PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold™ DNA Polymerase to cleave a TaqMan™ probe during PCR. Briefly, cDNA was generated from the samples of interest, *e.g.*, lung, ovary, colon, and breast normal and tumor samples, and used as the starting material for PCR amplification. In addition to the 5' and 3' gene-specific primers, a gene-specific oligonucleotide probe (complementary to the region being amplified) was included in the reaction (*i.e.*, the Taqman™ probe). The TaqMan™ probe includes the oligonucleotide with a fluorescent reporter dye covalently linked to the 5' end of the probe (such as FAM (6-carboxyfluorescein), TET (6-carboxy-4,7,2',7'-tetrachlorofluorescein), JOE (6-carboxy-4,5-dichloro-2,7-dimethoxyfluorescein), or VIC) and a quencher dye (TAMRA (6-carboxy-N,N,N',N'-tetramethylrhodamine) at the 3' end of the probe.

During the PCR reaction, cleavage of the probe separates the reporter dye and the

quencher dye, resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'-3' nucleolytic activity of the AmpliTaq™ Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control gene confirms efficient removal of genomic DNA contamination.

Tissue Distribution of Human 8099

A human tissue panel was tested revealing highest expression of human 8099 mRNA in congestive heart failure (CHF) heart, normal prostate, and brain (see Table 1, below).

TABLE 1.

Tissue Type	Mean	ß 2 Mean	∂∂ Ct	Expression
Artery normal	30.83	22.31	8.52	2.7241
Aorta diseased	32.77	22.32	10.45	0.7149
Vein normal	29.41	20.23	9.18	1.724
Coronary SMC	31.2	20.91	10.3	0.7932
HUVEC	32.16	21.38	10.78	0.5687
Hemangioma	32.86	19.66	13.21	0.1059
Heart normal	28.05	20.43	7.62	5.0834
Heart CHF	26.98	20.68	6.3	12.6914
Kidney	27.76	20.45	7.3	6.3238
Skeletal Muscle	29.7	22.17	7.53	5.4294
Adipose normal	34.16	20.59	13.56	0.0828
Pancreas	33.23	22.29	10.94	0.5108
primary osteoblasts	32	20.61	11.39	0.3726
Osteoclasts (diff)	30.9	17.55	13.35	0.0958
Skin normal	34.12	22.45	11.68	0.3058
Spinal cord normal	31.93	21.07	10.87	0.5362
Brain Cortex normal	28.4	22.34	6.06	14.9885
Brain Hypothalamus normal	29.68	22.35	7.34	6.1936
Nerve	32.96	22.25	10.72	0.5949
DRG (Dorsal Root Ganglion)	30.81	22.15	8.65	2.4808
Breast normal	31.91	21.14	10.77	0.5747
Breast tumor	32.73	20.93	11.81	0.2785
Ovary normal	30.41	19.82	10.6	0.6465

Ovary Tumor	28.36	19.06	9.31	1.5755
Prostate Normal	27.29	19.77	7.52	5.4482

Tissue Distribution of Human 46455

A human vessel and tissue panel was tested revealing highest expression of human 46455 mRNA in human umbilical vein endothelial cells (HUVEC), erythroid cells, normal artery, megakaryocytes, kidney, and CHF heart. 46455 was expressed at higher levels in lung tumor, breast tumor, and colon tumor versus normal lung, breast and colon tissues, indicating a possible role for 46455 in cellular proliferation disorders (see Table 2, below).

TABLE 2.

Tissue Type	Mean	ß 2 Mean	∂∂ Ct	Expression
Artery normal	28.05	24.09	2.67	157.6722
Aorta diseased	28.75	23.66	3.79	72.0429
Vein normal	27.75	21.72	4.75	37.1627
Coronary SMC	28.2	25.12	1.78	290.176
HUVEC	24.18	22.59	0.29	817.9021
Hemangioma	25.15	20.98	2.88	135.8419
Heart normal	26.44	21.82	3.33	99.4421
Heart CHF	25.54	21.09	3.15	112.6563
Kidney	25.98	21.49	3.2	108.8188
Skeletal Muscle	28.22	24.14	2.79	144.586
Adipose normal	28.38	22.21	4.88	33.9605
Pancreas	27.91	23.22	3.4	94.4045
primary osteoblasts	27.11	21.85	3.97	63.8133
Osteoclasts (diff)	23.64	18.8	3.55	85.3775
Skin normal	28.43	23.27	3.88	68.1567
Spinal cord normal	26.88	22.12	3.47	90.2456
Brain Cortex normal	26.42	23.4	1.73	301.452
Brain Hypothalamus normal	28.1	23.55	3.26	104.386
Nerve	28.59	23.88	3.43	92.7827
DRG (Dorsal Root Ganglion)	28.33	23.76	3.28	102.9489
Breast normal	27.31	22.32	3.7	76.9465
Breast tumor	26.47	22.11	3.07	119.0797
Ovary normal	26.59	22.16	3.13	113.8337
Ovary Tumor	28.47	21.84	5.33	24.8605
Prostate Normal	27.09	21.68	4.12	57.5117
Prostate Tumor	26.51	21.58	3.64	80.2141
Salivary glands	27.16	20.81	5.07	29.8733
Colon normal	26.3	20	5	31.1419
Colon Tumor	25.09	20.52	3.29	102.5927
Lung normal	26.02	19.75	4.98	31.6862
Lung tumor	25.09	21.31	2.48	178.6243
Lung COPD	25.26	19.71	4.26	52.193
Colon IBD	26.3	18.91	6.1	14.5786
Liver normal	27.66	21.8	4.57	42.101
Liver fibrosis	29.31	24.09	3.92	65.8351
Spleen normal	27.41	21.41	4.71	38.2075
Tonsil normal	25.23	19.32	4.63	40.5262
Lymph node normal	26.15	20.35	4.51	43.8889

Small intestine normal	28.23	21.73	5.22	26.8302
Skin-Decubitus	27.18	22.82	3.06	119.908
Synovium	28	21.12	5.59	20.6889
BM-MNC	26.13	19.32	5.51	21.9445
Activated PBMC	25.2	17.95	5.96	16.12
Neutrophils	24.45	19.5	3.65	79.66
Megakaryocytes	22.5	18.95	2.26	208.772
Erythroid	24.2	21.69	1.23	427.7975

Tissue Distribution of Human 53763

A human vessel and tissue panel was tested revealing highest expression of human 53763 mRNA in normal brain cortex, normal hypothalamus, prostate tumor, normal prostate, dorsal root ganglion, and normal breast tissue (see Table 3, below).

TABLE 3.

Tissue Type	Mean	ß 2 Mean	∂∂ Ct	Expression
Artery normal	40	22.41	16.07	0
Aorta diseased	40	22.05	16.44	0
Vein normal	40	19.75	18.74	0
Coronary SMC	35.16	21.86	11.79	0
HUVEC	40	20.41	18.08	0
Hemangioma	40	18.52	19.96	0
Heart normal	39.43	19.55	18.37	0
Heart CHF	40	18.98	19.5	0
Kidney	39.44	19.76	18.16	0
Skeletal Muscle	38.97	21.57	15.89	0
Adipose normal	40	20.09	18.4	0
Pancreas	38.91	20.84	16.56	0
primary osteoblasts	40	19.87	18.61	0
Osteoclasts (diff)	40	17.09	21.4	0
Skin normal	39.59	21.22	16.86	0
Spinal cord normal	31.72	20.14	10.07	0.9303
Brain Cortex normal	23.07	21.56	0.01	996.5403
Brain Hypothalamus normal	26.15	20.98	3.65	79.3844
Nerve	39.08	21.23	16.33	0
DRG (Dorsal Root Ganglion)	31.66	21.3	8.86	2.1596
Breast normal	27.25	20.41	5.33	24.9468
Breast tumor	40	20.02	18.46	0
Ovary normal	40	19.66	18.83	0
Ovary Tumor	40	19.7	18.79	0
Prostate Normal	29.68	19.32	8.85	2.1671
Prostate Tumor	28.14	19.95	6.67	9.8204
Salivary glands	40	18.97	19.52	0
Colon normal	39.09	17.8	19.78	0
Colon Tumor	40	18.63	19.86	0
Lung normal	40	17.49	21	0
Lung tumor	39.66	19.81	18.34	0
Lung COPD	40	17.97	20.52	0
Colon IBD	40	17.3	21.18	0

Liver normal	40	19.57	18.91	0
Liver fibrosis	40	21.34	17.15	0
Spleen normal	40	19.27	19.22	0
Tonsil normal	33.5	16.75	15.24	0.0258
Lymph node normal	38.61	18.4	18.7	0
Small intestine normal	36.56	19.96	15.08	0
Skin-Decubitus	39.43	20.41	17.51	0
Synovium	40	19.32	19.16	0
BM-MNC	40	18.21	20.27	0
Activated PBMC	38.88	17.5	19.88	0
Neutrophils	40	18.38	20.11	0
Megakaryocytes	40	18.09	20.39	0
Erythroid	40	21.23	17.25	0

Tissue Distribution of Human 67076

A human vessel panel was tested revealing highest expression of human 67076 mRNA in normal aorta, diseased artery, and static HUVEC (see Table 4, below).

TABLE 4.

Tissue Type	Mean	ß 2 Mean	∂∂ Ct	Expression
Aortic SMC	25.58	21.16	4.42	46.8762
Coronary SMC	29.11	24.36	4.76	36.906
Huvec Static	23.55	20.59	2.96	128.0696
Huvec LSS	23.41	20.06	3.35	98.073
H/Adipose/MET 8	27.7	20.51	7.18	6.8723
H/Artery/Normal/Carotid/CLN 595	26.82	19.34	7.48	5.6014
H/Artery/Normal/Carotid/CLN 598	28.79	20.41	8.37	3.0226
H/Artery/normal/NDR 352	29.41	21.68	7.73	4.7102
H/IM Artery/Normal/AMC 73	32.65	23.77	8.88	2.1152
H/Muscular Artery/Normal/AMC 236	29.2	23.34	5.87	17.1577
H/Muscular Artery/Normal/AMC 254/	29.68	22.56	7.13	7.1393
H/Muscular Artery/Normal/AMC 259	29.63	22.25	7.37	6.0452
H/Muscular Artery/Normal/AMC 261	30.12	22.67	7.45	5.7191
H/Muscular Artery/Normal/AMC 275	30.2	24.2	6	15.6792
H/Aorta/Diseased/PIT 732	30.73	22.36	8.38	3.0121
H/Aorta/Diseased/PIT 710	29.6	23.14	6.46	11.3199
H/Aorta/Diseased/PIT 711	29.35	22.63	6.72	9.4531
H/Aorta/Diseased/PIT 712	28.77	22.02	6.75	9.2585
H/Artery/Diseased/iliac/NDR 753	26.11	19.41	6.71	9.585
H/Artery/Diseased/Tibial/PIT 679	29.82	20.34	9.47	1.4101
H/Vein/Normal/SaphenousAMC 107	31.66	21.07	10.59	0.6488
H/Vein/Normal/NDR 239	33.13	21.65	11.49	0.3477
H/Vein/Normal/Saphenous/NDR 237	29.71	20.59	9.12	1.7972
H/Vein/Normal/PIT 1010	28.34	22.05	6.3	12.6914
H/Vein/Normal/AMC 191	28.64	22.15	6.49	11.164
H/Vein/Normal/AMC 130	27.41	21.27	6.14	14.1309
H/Vein/Normal/AMC 188	30.56	24.09	6.46	11.3199
H/Vein/Normal/AMC 196	29.89	20.93	8.96	2.008
H/Vein/Normal/AMC 211	32.55	23.52	9.03	1.9196
H/Vein/Normal/AMC 214	30.93	22.99	7.95	4.058
M/Artery/Diseased/CAR 1174	24.56	23.05	1.5	352.3302
M/Artery/Diseased/CAR 1175	24.98	19.89	5.09	29.2585

M/Aorta/Normal/PRI 286	25.52	18.68	6.84	8.7288
M/Artery/Normal/PRI 324	25.13	20.65	4.48	44.8111
M/Aorta/Normal/PRI 264	24.14	24.74	-0.6	1515.7166
M/Artery/Normal/PRI 320	24.93	20.29	4.64	40.1071
M/Vein/Normal/PRI 328	26.67	20.04	6.63	10.0965
HUVEC Vehicle	26.64	21	5.63	20.1232
HUVEC Mev	25.54	20.3	5.25	26.3692
HAEC Vehicle	25.7	20.66	5.04	30.2903
HAEC Mev	27.84	22.41	5.43	23.1957

Tissue Distribution of Human 67102

A human tissue panel was tested revealing highest expression of human 67102 mRNA in normal kidney tissue and diseased artery (see Table 5, below).

TABLE 5.

Tissue Type	Mean	ß 2 Mean	∂∂ Ct	Expression
Aortic SMC	28.34	21.88	6.47	11.2807
Coronary SMC	29.98	23.11	6.88	8.5196
Huvec Static	27.55	21.41	6.14	14.18
Huvec LSS	27.72	21.12	6.59	10.3444
H/Adipose/MET 8	30.56	20.57	9.99	0.9834
H/Artery/Normal/Carotid/CLN 595	31.42	20.3	11.13	0.4478
H/Artery/Normal/Carotid/CLN 598	32.23	21.69	10.54	0.6717
H/Artery/normal/NDR 352	31.34	22.44	8.9	2.0933
H/IM Artery/Normal/AMC 73	33.46	23.98	9.48	1.4003
H/Muscular Artery/Normal/AMC 236	30.48	23.52	6.96	8.0321
H/Muscular Artery/Normal/AMC 247	33.9	24.07	9.82	1.1025
H/Muscular Artery/Normal/AMC 254/	31.12	23.43	7.68	4.8594
H/Muscular Artery/Normal/AMC 259	30.47	23.07	7.4	5.9208
H/Muscular Artery/Normal/AMC 261	31.32	22.92	8.4	2.9501
H/Muscular Artery/Normal/AMC 275	31.31	24.78	6.53	10.8212
H/Aorta/Diseased/PIT 732	31.73	22.76	8.97	1.9942
H/Aorta/Diseased/PIT 710	30.33	23.36	6.97	7.9767
H/Aorta/Diseased/PIT 711	31.02	23.3	7.72	4.7265
H/Aorta/Diseased/PIT 712	30.57	22.71	7.86	4.3043
H/Artery/Diseased/iliac/NDR 753	27.22	20.07	7.15	7.041
H/Artery/Diseased/Tibial/PIT 679	32	21.19	10.81	0.557
H/Vein/Normal/SaphenousAMC 107	31.57	22.08	9.49	1.3859
H/Vein/Normal/NDR 239	33.44	22.16	11.28	0.4021
H/Vein/Normal/Saphenous/NDR 237	31.32	21.01	10.31	0.7877
H/Vein/Normal/PIT 1010	29.86	22.36	7.5	5.5243
H/Vein/Normal/AMC 191	30.36	22.53	7.84	4.3796
H/Vein/Normal/AMC 130	30.08	22.32	7.75	4.6293
H/Vein/Normal/AMC 188	32.93	25.01	7.92	4.129
H/Vein/Normal/AMC 196	32.24	21.61	10.64	0.6288
H/Vein/Normal/AMC 211	36.16	23.51	12.65	0
H/Vein/Normal/AMC 214	35.59	24	11.6	0
M/Artery/Diseased/CAR 1175	29.73	21.84	7.89	4.2011
M/Aorta/Normal /543	34.84	29.17	5.67	19.6408
M/Artery/Diseased/CAR 1174	31.11	26.59	4.52	43.5857

M/Pancreas/PRI 2	32.48	26.33	6.15	14.082
M/Kidney/Normal/MPI 88	30.23	26.84	3.38	96.0547
M/Kidney/Normal/MPI 282	29.34	25.94	3.4	95.0612
HUVEC Vehicle	29.25	21.45	7.8	4.4871
HUVEC Mev	28.16	20.87	7.29	6.3899
HAEC Vehicle	28.14	21.16	6.97	7.9491
HAEC Mev	29.61	22.66	6.95	8.088

In addition, a human vessel panel was tested, which revealed high expression of human 67102 mRNA in normal artery, HUVEC, coronary smooth muscle cells, diseased aorta, and normal hypothalamus (see Table, 6, below).

TABLE 6.

Tissue Type	Mean	ß 2 Mean	∂∂ Ct	Expression
Artery normal	27.32	21.75	5.57	21.0505
Aorta diseased	28.27	21.71	6.55	10.6353
Vein normal	30.38	19.83	10.55	0.6693
Coronary SMC	28.61	22.23	6.38	12.0485
HUVEC	26.32	20.32	6	15.5709
Hemangioma	25.91	19.07	6.83	8.7895
Heart normal	27.16	19.98	7.17	6.9441
Heart CHF	27.2	19.06	8.14	3.545
Kidney	25.54	19.59	5.96	16.12
Skeletal Muscle	30.52	21.5	9.03	1.9196
Adipose normal	30.11	19.95	10.15	0.8771
Pancreas	29.57	21.23	8.33	3.1076
primary osteoblasts	28.09	19.85	8.23	3.3191
Osteoclasts (diff)	29.79	17.02	12.77	0.1432
Skin normal	29.31	21.41	7.89	4.2011
Spinal cord normal	28.3	20.36	7.93	4.0863
Brain Cortex normal	28.25	22.04	6.21	13.5084
Brain Hypothalamus normal	28.93	21.49	7.44	5.7589
Nerve	28.34	21.3	7.04	7.5989
DRG (Dorsal Root Ganglion)	29.16	21.11	8.04	3.7994
Breast normal	27.81	20.47	7.34	6.1508
Breast tumor	29.08	20.41	8.68	2.4466
Ovary normal	26.44	19.7	6.74	9.3878
Ovary Tumor	30.93	19.6	11.34	0.3871
Prostate Normal	28.11	19.48	8.63	2.5241
Prostate Tumor	27.68	19.68	8	3.9063
Salivary glands	28.9	19.18	9.71	1.194
Colon Tumor	27.98	18.82	9.16	1.742
Lung normal	26.96	17.4	9.56	1.3202
Lung tumor	27.82	19.64	8.19	3.4361
Lung COPD	26.38	17.66	8.72	2.3633
Colon IBD	28.27	17.29	10.98	0.4934
Liver normal	29.14	19.58	9.56	1.3248
Liver fibrosis	29.89	21.08	8.8	2.2358
Spleen normal	26.95	19.09	7.86	4.3193
Tonsil normal	25.01	16.8	8.21	3.3654
Lymph node normal	26.3	18.22	8.09	3.6828
Small intestine normal	29.03	19.59	9.45	1.4347

Skin-Decubitus	27.66	20.32	7.34	6.1722
Synovium	28.22	19.23	8.98	1.9804
BM-MNC	29.57	18.46	11.12	0.4509
Activated PBMC	28.38	17.25	11.14	0.4447
Neutrophils	27.43	18.4	9.04	1.8997
Megakaryocytes	26.72	17.88	8.84	2.1822
Erythroid	31.52	21.26	10.26	0.8183
Colon normal	30.07	19.25	10.82	0.5551

Tissue Distribution of Human 44181

A human vessel panel was tested revealing highest expression of human 44181 mRNA in LSS HUVEC (see Table 7, below).

TABLE 7

Tissue Type	Mean	ß 2 Mean	∂∂ Ct	Expression
Static Huvec	25.37	19.18	6.19	13.697
LSS Huvec	25.7	20.02	5.68	19.4377
Aortic SMC	28.75	20.32	8.43	2.9095
Coronary SMC	28.52	21.2	7.31	6.3019
H/Adipose/MET 9	36.07	18.41	17.66	0
Diseased Heart /PIT 1	29.28	21.15	8.13	3.5697
H/Artery/Normal/Carotid/CLN 595	37.9	18.32	19.59	0
H/Artery/Normal/Carotid/CLN 598	39.97	19.49	20.48	0
H/Artery/normal/NDR 352	40	20.2	19.8	0
H/Artery/Normal/AMC 150	40	22.27	17.73	0
H/Artery/Normal/AMC 73	40	23.84	16.16	0
IMA / AMC 247	39.73	22.79	16.95	0
IMA / AMC 254	33.79	22.23	11.56	0.3324
IMA / AMC 259	33.68	21.12	12.56	0.1656
IMA / AMC 261	34.73	21.23	13.5	0.0863
IMA / AMC 275	40	24.52	15.48	0
IMA / AMC 279	30.89	22.41	8.48	0
H/Artery/Diseased/iliac/NDR 753	36.59	18.43	18.16	0
H/Artery/Diseased/Tibial/PIT 679	40	19.84	20.16	0
Aorta/Diseased/PIT 732	34.74	21.32	13.41	0.0916
Aorta/Diseased/ PIT 710	33.04	22.48	10.56	0.6624
Aorta/Diseased/PIT 711	31.89	22.09	9.8	1.1218
Aorta/Diseased/ PIT 712	32.92	22.09	10.84	0.5474
H/Vein/Normal/Saphenous/NDR 721	32.66	16.82	15.83	0.0172
H/Vein/Normal/SaphenousAMC 107	40	20	20	0
H/Vein/Normal/NDR 239	40	20.61	19.39	0
H/Vein/Normal/Saphenous/NDR 237	40	19.1	20.9	0
H/Vein/Normal/NDR 235	40	21.34	18.66	0

H/Vein/Normal/MPI 1101	33.56	19.59	13.98	0.0621
HMVEC/Vehicle/24 hr	30.04	17.84	12.2	0.2125
HMVEC/Mev/24hr/1X	29.77	18	11.76	0.2883
HMVEC/MEV/24HR/2.5X	30.32	18.67	11.65	0.3112
HMVEC/MEV/48HR/1X	31.58	18.8	12.79	0.1417
HMVEC/MEV/48HR/2.5X	31.77	18.37	13.4	0.0922
HUVEC/Vehicle/24 hr	30.5	18.15	12.36	0.1909
HUVEC/Mev/24hr/1X	30.28	17.52	12.76	0.1442
HUVEC/MEV/24HR/2.5X	29.35	19.18	10.18	0.865
HUVEC/MEV/48HR/1X	35.68	21.54	14.14	0
HUVEC/MEV/48HR/2.5X	34.7	23	11.7	0.3016

Tissue Distribution of Human 67084

A human vessel panel was tested revealing highest expression of human 67084 mRNA in HUVEC, LSS HUVEC, and coronary smooth muscle cells (see Table 8, below).

TABLE 8.

Tissue Type	Mean	ß 2 Mean	∂∂ Ct	Expression
Aortic SMC	25.92	19.23	6.7	9.6517
Coronary SMC	26.59	20.36	6.23	13.3224
Huvec Static	23.39	18.5	4.88	33.843
Huvec LSS	24.31	18.32	5.99	15.7883
H/Adipose/MET 9	26.4	18.46	7.94	4.0721
H/Artery/Normal/Carotid/CLN 595	26.83	18.84	8	3.9198
H/Artery/Normal/Carotid/CLN 598	28.49	20.16	8.34	3.0968
H/Artery/normal/NDR 352	27.12	20.32	6.8	8.9432
H/IM Artery/Normal/AMC 73	31.48	23.36	8.12	3.607
H/Muscular Artery/Normal/AMC 236	30.93	23.56	7.38	6.0243
H/Muscular Artery/Normal/AMC 247	33.77	24.84	8.92	2.0645
H/Muscular Artery/Normal/AMC 254/	30.69	23.68	7	7.7855
H/Muscular Artery/Normal/AMC 259	29.9	22.12	7.78	4.5497
H/Muscular Artery/Normal/AMC 261	29.93	21.13	8.8	2.2436
H/Muscular Artery/Normal/AMC 275	30.29	22.97	7.33	6.2367
H/Aorta/Diseased/PIT 732	29.02	21.35	7.67	4.8932
H/Aorta/Diseased/PIT 710	31.36	22.8	8.56	2.6496
H/Aorta/Diseased/PIT 711	31.31	22.6	8.71	2.3963
H/Aorta/Diseased/PIT 712	31.4	22.48	8.92	2.0645
H/Artery/Diseased/iliac/NDR 753	25.37	17.73	7.64	4.996
H/Artery/Diseased/Tibial/PIT 679	28.55	19.45	9.11	1.816
H/Vein/Normal/SaphenousAMC 107	29.48	21.11	8.38	3.0121
H/Vein/Normal/Saphenous/NDR 237	28.67	19.86	8.8	2.2358
H/Vein/Normal/PIT 1010	28.31	20.55	7.76	4.5973
H/Vein/Normal/AMC 191	29.25	20.77	8.47	2.8104
H/Vein/Normal/AMC 130	28.32	20.45	7.88	4.2598
H/Vein/Normal/AMC 188	31.68	24.61	7.06	7.4943
H/Vein/Normal/NDR 239	35.65	29.23	6.42	0
HUVEC Vehicle	26.86	20.14	6.71	9.5188
HUVEC Mev	25.83	18.52	7.3	6.3238
HAEC Vehicle	26.57	19.64	6.94	8.1443
HAEC Mev	27.81	21.13	6.67	9.7864

EXAMPLE 3: EXPRESSION OF RECOMBINANT 67076, 67102, 44181, 67084FL, or 67084alt POLYPEPTIDE IN BACTERIAL CELLS

In this example, human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt is fused to GST and this fusion polypeptide is expressed in *E. coli*, *e.g.*, strain PEB199. Expression of the GST-PLTR fusion polypeptide in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

EXAMPLE 4: EXPRESSION OF RECOMBINANT 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt POLYPEPTIDES IN COS CELLS

To express the human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire PLTR polypeptide and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant polypeptide under the control of the CMV promoter.

To construct the plasmid, the human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA).

Preferably the two restriction sites chosen are different so that the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5α, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the IC54420 polypeptide is detected by radiolabelling (^{35}S-methionine or ^{35}S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with ^{35}S-methionine (or ^{35}S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the human 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt polypeptide is detected by radiolabelling and immunoprecipitation using a 8099, 46455, 54414, 53763, 67076, 67102, 44181, 67084FL, or 67084alt -specific monoclonal antibody.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.